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# Restriction fragment length polymorphism in sorghum (*Sorghum bicolor* (L.) Moench): characterization of genetic diversity and quantitative trait loci

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Restriction fragment length polymorphism in sorghum  
[*Sorghum bicolor* (L.) Moench] : Characterization of  
genetic diversity and quantitative trait loci

by

Dario Ahnert

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of the  
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## GENERAL INTRODUCTION

### Literature Review

Sorghum [*Sorghum bicolor* (L.) Moench] is the leading cereal grain in the rainfed areas of the semiarid tropics and subtropics (Poehlman, 1987). An important advantage of sorghum is its tolerance to drought and heat stress. Due to this characteristic, sorghum is cultivated in the southern and central Great Plains region of the U.S. considered to be too hot and dry for commercial maize production.

It is believed that sorghum was first domesticated in the north-east quadrant of Africa (Doggett, 1988). From there it spread to central and southern Africa, Western Africa, India, and to other regions of the World (Klier, 1988). The first sorghum introduced into U.S. was "guinea corn" brought from West Africa with the slave-trade (Doggett, 1988). Around the year of 1955, a total of 13,764 accessions was in the U.S. collection. By the end of the last decade (1989), the number of accessions increased to 32,605 (Duncan et al., 1991).

The varieties grown in the early stage of sorghum cultivation in the U.S. were tall with late flowering and grain maturity (Poehlman, 1987). They were considered inadequate for mechanized harvesting and too late for this temperate region. The solution for these problems came with the discovery of mutations for early maturity (Quinby and Karper, 1945; Quinby, 1966) and dwarfness (Quinby and Karper,

1954). Short stature varieties amenable to be mechanized harvesting with early maturity were selected by farmers and became widely grown. It was found that most of the early varieties traced to germplasm from the groups Kafir, Milo, Hegari, and Feterita (Webster, 1976).

In the 1950s, the traditional varieties were substituted by  $F_1$  hybrid cultivars (Duncan et al., 1991). Development of hybrid seeds on an economical basis became possible with the discovery of cytoplasmic male sterility and the fertility restoration system in sorghum. Cytoplasmic male sterility is believed to be the result of incompatibility between the Milo cytoplasm and the Kafir nuclear factors (Miller, 1979). With the adoption of cytoplasmic male sterility for the obtention of hybrid seeds, breeding efforts were directed toward obtaining parental lines with good sterility-fertility restoration and good combining ability. Great efforts have been made in the production of male sterile A-lines (lines with cytoplasm male-sterile and non-restorer nuclear genes), restorer R-lines (lines with cytoplasm male-sterile or normal and homozygous for nuclear restorer genes), and B-lines (lines with cytoplasm male-fertile and non-restorer nuclear factors). For the development of A-lines there are alternative sources of cytoplasm designated A1, A2, A3, and A4. Source A1 contains Milo cytoplasm and the remaining contains cytoplasm from lines generated in the "Sorghum Conversion Program". However, the Milo (A1) cytoplasm continues to be the most widely used because the others often revert to male fertility in unfavorable environments and have some adverse agronomic



characteristics (Poehlman, 1987; Duncan et al., 1991).

The use of male-sterility for hybrid seed production has led to the narrowing of the germplasm pool especially among A-lines (Duncan et al., 1991). Breeders concerned with the narrow genetic base of the U.S. sorghum industry proposed the establishment of the "Sorghum Conversion Program" (Miller, 1979; Poehlman, 1987). The main objective of this program is to convert tropical varieties into short, day-neutral genotypes adapted to temperate climates (Miller, 1979). Duncan et al. (1991) reported that after the "Sorghum Conversion Program" released, in 1974, the first advanced parental cultivars with converted lines in their pedigree, the genetic base has slowly widened. Despite the large amount of germplasm available in the germplasm banks and the good results of the conversion program, the level of genetic diversity continues narrow (Webster, 1976; Poehlman, 1987; Duncan et al., 1991). The main causes of the limited use of germplasm are: a) the amount of time and resources required to convert the introductions from photoperiod sensitive to insensitive (Duncan et al., 1991) and; b) the almost exclusive use of Kafir nuclear genes and Milo cytoplasm for the production of  $F_1$  hybrids (Webster, 1976).

Compared with maize, sorghum is poorly characterized in terms of basic genetics. It is a diploid species with  $2n = 2x = 20$ . Around 200 Mendelian genes have been identified, but, very few linkage groups have been established (Doggett, 1988). Maturity and plant height have been extensively characterized through genetic analyses and inheritance studies. However, little genetic information is

available for other morphological traits.

Maturity varies with the genotype and is influenced by both temperature and photoperiod (Quinby, 1967). Four independent loci ( $ma_1$ ,  $ma_2$ ,  $ma_3$ , and  $ma_4$ ) with many alleles at each locus control maturity and may also influence growth and number of leaves (Quinby, 1967, 1975 ; Doggett, 1988). Genotypes adapted to tropical zones have dominant alleles at the four loci while genotypes adapted to temperate zones have one or more recessive alleles at any of these loci (House, 1985). Sorghum is considered a short-day plant. It maintains the vegetative bud throughout the growing phase until the day length became short enough, where upon it achieves the critical photoperiod for flower buds to develop. The range of flowering within a genotype varies from 10 or 12 days. The last plants that emerge from the soil are usually the latest to flower (Quinby, 1967).

Plant height is influenced by node number, internode, peduncle and panicle length, and growing conditions (Doggett, 1988). Four independently inherited genes ( $dw1$ ,  $dw2$ ,  $dw3$ , and  $dw4$ ) control stem internode length (Quinby and Karper, 1954). Tallness (long internode) is partially dominant to dwarfness; with the dwarfing effect being brachytic in nature (House, 1985). Plants with four dominant genes may reach four meters in height. Changing of any one of the four dominant to a recessive gene decreases the height by 50 cm. However, if one or more of the four loci already have a recessive allele, change of an additional dominant to a recessive may result in a smaller plant height reduction (House, 1985).

The discovery of mutations for early maturity, dwarfness, and male-sterility in sorghum were crucial for the establishment of the U.S. sorghum industry in an economical basis. Sorghum breeders have used these mutations for the production of F<sub>1</sub> hybrids with superior agronomic performance. However, new advances in the genetic improvement of this crop have been considered slower than the expected rate (Kramer, 1987). It has been suggested that the incorporation of molecular techniques and novel breeding approaches could help to accelerate the improvement of sorghum (Duncan et al., 1991).

Restriction fragment length polymorphisms (RFLPs) were first proposed for genetic studies of plant species by Tanksley (1983) and Burr et al. (1983). This class of molecular markers is selectively neutral, ubiquitous, and usually displays codominant inheritance (Murray et al., 1988). Molecular marker studies (mainly RFLPs) have expanded the genetic characterization of sorghum. Much genetic diversity was found in wild than in cultivated sorghum for isozyme (Morden et al., 1990; Aldrich et al., 1992) and RFLPs variations (Aldrich and Doebley, 1992). These studies compared variability in wild and cultivated sorghum and pointed to the domestication process as the reason for the reduction of variability in the cultivated forms. Subsequent studies in cultivated sorghum based on RFLPs and random amplified polymorphic DNA (RAPDs) reported low (Tao et al., 1993) to moderate (Vierling et al., 1994) levels of genetic diversity. However, both studies used small number of genotypes and mostly unmapped RFLP markers.

The feasibility of RFLP mapping in sorghum using maize genomic probes was demonstrated by Hulbert et al. (1990). Of the 105 probes tested, 104 hybridized strongly to sorghum DNA. The level of polymorphism observed in sorghum allowed the establishment of eight clusters or linkage groups. Comparison of these linkage groups to that of maize revealed that most RFLP loci linked in sorghum were also linked in maize. Whitkus et al. (1992) using 7 isozyme loci and 85 maize genomic probes identified 13 linkage groups in sorghum and showed that many of these linkage groups are conserved between maize and sorghum. Recently, Pereira et al. (1994) developed the most complete linkage map of sorghum by using maize genomic probes (57), cDNA maize probes (134), and sorghum genomic probes (10). Ten linkage groups were identified with 201 markers. Also, a considerable level of genome conservation was observed between sorghum and maize.

By using RFLPs, QTL affecting the expression of morphological and maturity traits were identified in an  $F_2$  sorghum population (Pereira and Lee, 1995). Four *unlinked* genomic regions were identified for plant height. These regions accounted for 63.4% of the phenotypic variation for height in a multiple QTL model and may correspond to the *Dw* loci. These regions were also proposed to be orthologous to those affecting plant height in maize. Four QTL were identified for number of tillers, three for leaf length, two for leaf width, three for stalk circumference, and two for maturity (Pereira, 1993). These QTL individually accounted for 8 to 27% of the

phenotypic variation in single QTL models. Six QTL were identified for panicle length, five for seed branch length, two for length of sterile portions of the seed-branch, six for peduncle diameters, three for number of seed-branches per panicle, and three for 100-seed weights (Pereira et al., 1995). In a larger and more divergent  $F_2$  population, six QTL were detected for plant height and three for flowering date explaining 71% and 87%, respectively, of the phenotypic variation (Lin et al., 1995). In  $F_7$  recombinant inbred lines, three major regions were identified for drought resistance (Xu et al., 1994). These QTL accounted for 8 to 37% of the phenotypic variation of a trait in single QTL models. In all these QTL studies, the parent with the larger phenotypic mean usually contributed alleles for increased value of that trait. Gene action varied from additive to overdominance. Usually, significantly correlated traits had QTL in common genomic regions indicating linkage and/or pleiotropy.

The objectives of this research were accomplished by using RFLPs to study genetic diversity and quantitative trait variation in two different sets of sorghum genotypes. One set was composed of 105 elite inbred lines (47 B-lines and 58 R-lines) of different germplasm sources and the other of 152  $F_{2,3}$  lines. In the R- and B-lines set, the main objective was to define the level of genetic diversity and genetic relationships within these elite inbred lines. Another important issue in this study was the comparison between the RFLP-based genetic similarity and the estimates of coancestry coefficients ( $f$ ) within R and B-lines. In the 152  $F_{2,3}$  lines set,

the main objective was to evaluate this population to characterize QTL affecting plant height, height to the uppermost node, peduncle length, panicle length and width, leaf length and width, number of nodes and tillers, stalk circumference and maturity. From these data, other important questions in quantitative trait inheritance in sorghum could be investigated. These include comparison of QTL mapping in the  $F_{2,3}$  to those obtained previously in the  $F_2$  plants, comparison of QTL map location for plant height in sorghum and maize, and the association between QTL affecting height and qualitative genes controlling this trait.

#### **Dissertation Organization**

This dissertation includes three manuscripts preceded by a General Introduction section. The first manuscript reports on the genetic diversity and relationships among sorghum elite inbred lines. The second manuscript reports on genetic mapping of quantitative trait loci affecting plant height in  $F_{2,3}$  and  $F_2$  sorghum population and comparative mapping with maize. The third manuscript reports on genetic effects and locations of quantitative trait loci affecting morphological traits in sorghum (plant height to the uppermost node, peduncle length, panicle length and width, leaf length and width, stalk circumference, number of tillers and nodes, and flowering date). These manuscripts are followed by a General Conclusion section. The references cited in the General Introduction and General Conclusions sections are listed in the General References section. The Appendix includes additional information pertinent to the main text.

## MOLECULAR GENETIC DIVERSITY AMONG ELITE SORGHUM INBRED LINES

A paper accepted by *Crop Science*

D. Ahnert<sup>1</sup>, D. Austin<sup>1</sup>, M. Lee<sup>1\*</sup>, C. Livini<sup>1</sup>, W.L Woodman<sup>1</sup>, S.J. Openshaw<sup>2</sup>, J.S.C. Smith<sup>2</sup>, K. Porter<sup>2</sup>, and G. Dalton<sup>2</sup>

### Abstract

Restriction fragment length polymorphisms (RFLPs) and pedigree data were used to investigate the genetic relationships in a group of 58 R- and 47 B- elite sorghum inbred lines. The objectives of this study were to I) verify the level of genetic variation for RFLPs in these lines, II) determine estimates of genetic similarity (GS) based on RFLPs and pedigree information for R- and B-lines and, III) examine the agreement between RFLP-based GS and coancestry coefficient ( $f$ ) for related ( $f > 0$ ) pairs of inbreds. R-lines, derived mainly from Feterita and Zera-zera, and B-lines, mostly from Kafir were analyzed for RFLPs with 104 DNA probes. A total of 326 RFLP bands was observed, with 276 common to R- and B-lines, 32 unique to R-lines, and 18 unique to B-lines. On average, 3.6 patterns per clone-enzyme combination were found for R-lines and 3.0 for B-lines. Average GS based on RFLPs was 0.67 for R-lines and 0.76 for B-lines indicating more diversity within the R- group. The coancestry coefficient ( $f$ ) was low in both groups of lines.

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The average for R-lines was 0.08 and for B-lines 0.07. Cluster analysis of GS estimates from the entire set (105) of inbreds revealed separate groupings for R- and B-lines agreeing with parental types, pedigree, and the classification system utilized by breeders. R-lines clustered into two main groups, one derived mainly from Feterita and the other from Zera-zera. B-lines were grouped into different sub-clusters, one composed mostly of lines derived from Martin and Redlan, and the others with the remaining lines. Principal component analysis showed results similar to cluster analysis. R-lines formed three loose clusters: one with Feterita, another with Zera-zera, and the third with the remaining sources. In contrast, most of the B-lines were grouped in one tight cluster. There was a moderate positive correlation between GS and  $f$  for 825 related ( $f > 0$ ) pairs of R-lines ( $r = 0.46$ ) and for 549 related pairs of B-lines ( $r = 0.43$ ) suggesting that RFLP data can help quantify the degree of relatedness in elite sorghum germplasm.

### Introduction

Cultivated sorghum (*Sorghum bicolor* (L.) Moench), commonly known as sorghum or milo in the U.S., is an important cereal crop worldwide (Doggett, 1988). Due to its tolerance to drought and heat stress (Poehlman, 1987), sorghum is cultivated in the southern and central Great Plains of the U.S., areas considered to be too hot and too dry for commercial maize production.  $F_1$  sorghum hybrids produced through the exploitation of genetic male sterility are grown as cultivars.



Three different types of parental lines are used for hybrid seed production: I) R-lines (lines with cytoplasm male-sterile or normal and homozygous for nuclear restorer factors); II) A-lines (lines with cytoplasm male-sterile and non-restorer nuclear factors); and III) B-lines (lines with cytoplasm male-fertile and non-restorer nuclear factors). Genetic diversity of these lines is considered narrow according to pedigree records, especially among A-lines (Poehlman, 1987; Duncan et al., 1991).

However, a comprehensive study measuring the level of diversity is not available. Much more genetic diversity was found in wild than in cultivated sorghum for isozyme (Morden et al., 1990; Aldrich et al., 1992) and restriction fragment length polymorphism (RFLP) variation (Aldrich and Doebley, 1992). These studies compared variability in wild and cultivated sorghum and pointed to the domestication process as the reason for the reduction of variability in the cultivated forms.

Subsequent studies in cultivated sorghum based on RFLPs and random amplified polymorphic DNA (RAPDs) reported low (Tao et al., 1993) to moderate (Vierling et al., 1994) levels of genetic diversity. However, both studies analyzed few genotypes and their RFLP markers were mostly unmapped.

Further exploitation of genetic diversity based on RFLPs may be facilitated by the development of a dense RFLP linkage map in sorghum (Hulbert et al., 1990; Whitkus et al., 1992; Berhan et al., 1993; Pereira et al., 1994). With the current number of mapped markers in sorghum, a more representative sample of regions throughout the genome can be investigated.

Comprehensive studies of genetic diversity based on DNA markers in elite inbred lines have been reported in maize (*Zea mays* L., Lee et al., 1989; Smith et al., 1990, 1991; Melchinger et al., 1991) and barley (*Hordeum vulgare* L.; Melchinger et al., 1994), but a survey of genetic diversity as assessed by RFLPs has not been reported for a large sample of elite sorghum germplasm. The objectives of this study were to I) investigate the level of genetic variation for RFLPs in a sample of 58 R- and 47 B- elite sorghum inbred lines, II) determine estimates of genetic similarity (GS) based on RFLPs and pedigree information within R- and B-lines, and III) examine the agreement between RFLP-based GS and coancestry coefficient ( $f$ ) for related ( $f > 0$ ) pairs of sorghum inbreds.

### **Materials and Methods**

#### **Genetic material**

One hundred and five private elite sorghum inbred lines (Table 1) including 58 R- and 47 B-lines derived from different germplasm sources were investigated for pedigree and RFLP data. These inbreds were mostly grain sorghum, but including a few forage types.

The R-lines originated mainly from Feterita and Zera-zera germplasm. In addition, some R-lines originated from Norghum, Graingrass, Yellow germplasm, forage sorghum (025 and 039), and complex sources (041). The B-lines originated mainly from Kafir germplasm. In this group, there were also lines tracing to Martin, Kaoling, Zera-zera, Redlan, Wheatland, Plainsman, Yellow germplasm and complex

sources.

#### **RFLP data collection**

For each inbred line, leaves from 10 plants grown in the field to six-leaf stage were collected, lyophilized, ground to a powder, and stored in vials at -20°C. The procedures for DNA isolation, separate digestion with restriction enzymes *EcoRI* and *HindIII*, electrophoresis on 0.7% agarose gel, Southern transfer to nylon membranes, hybridization with P<sup>32</sup> labeled probes, and autoradiography were performed as described by Veldboom et al. (1994). Digested DNA of the 105 lines was loaded on gels including one F<sub>1</sub> maize line as control and three lanes of a molecular weight standard composed of fragments of 1.1, 2.0, 2.3, 3.5, 3.7, 4.2, 5.6, 6.6, 8.1, 9.6, 13.2, and 23.1 Kb, derived from various restriction digests of lambda. In addition, high (24 Kb) and low (2.0 Kb) molecular weight standards were loaded together with each lane.

The 104 RFLP clones used as probes originated from maize genomic, sorghum genomic, and maize cDNA libraries (Table 2). The maize genomic clones are from collections of clones developed at Brookhaven National Laboratories (BNL), Native Plants Inc. (NPI), Pioneer Hi-bred International (PIO) and University of Missouri-Columbia (UMC). The sorghum genomic clones (ISU027 and ISU031) and maize cDNA clones were developed at Iowa State University (ISU) (Pereira et al., 1994).

RFLP patterns on the autoradiograms were determined for each clone-

enzyme combination (CEC). The entire set of inbreds was visually analyzed from the autoradiograms to quantify the number of different bands (different molecular weight categories) per CEC. The molecular weight of each band was estimated by its approximate migration distance in comparison with the lambda markers. Once the number of bands was defined, an identification number was assigned to each full-intensity band; reduced-intensity bands were rejected. For further analysis, the data were binary coded with band presence coded by 1 and absence by 0.

CECs were considered to have a single band if just a single band per line was present in the autoradiogram. Those generating more than one band for at least one line were considered to have multiple bands. The number of RFLP patterns per CEC was defined by counting the different combinations of bands observed across the entire set of lines for each CEC.

### **Statistical analysis**

For the statistical analysis, both monomorphic and polymorphic bands were included. GS estimates were calculated between pairs of inbred lines according to the following equation (Nei and Li, 1979):

$$GS_{ij} = 2N_{ij} / (N_i + N_j) \text{ where;}$$

$N_{ij}$  is the total number of bands common between two inbreds,  $i$  and  $j$ , and  $N_i$  and  $N_j$  are the total number of bands in  $i$  and  $j$ , respectively, considering all CEC employed.

GS values reflect the proportion of bands in common between two inbred lines.

Thus, GS value of 1 indicates complete similarity between two lines while a GS

value of 0 indicates maximum divergence between two lines.

Estimates of  $f$  were calculated for pairs of inbred lines based on available pedigree information according to the method described by Falconer (1989). To calculate  $f$ , the following assumptions were considered (Melchinger et al., 1991): I) all lines in a pedigree (parental and ancestral lines) were homozygous and homogeneous; II) lines without known common parentage were considered to be unrelated ( $f = 0$ ); and III) any inbred line derived from a cross obtained half of its genes from each parent. The  $f$  values were not calculated for all pairs of line combinations because of unavailable pedigree information. Line combinations with an unknown  $f$  were not considered for further analysis. Pearson correlation coefficients ( $r$ ) were calculated for the regression of GS on  $f$  for R- and B-line combinations where  $f > 0$  (Steel and Torrie, 1980).

The UPGMA (unweighted pair-group method, arithmetic average) clustering procedure was performed on the GS estimates of the 105 lines to reveal hierarchical groupings (Mumm and Dudley, 1994).

Principal component analysis (PCA) was performed on the correlation matrices of the RFLP data of the R- and B- parental lines for verification of the pattern of association among germplasm groups. All calculations and graphical representation were performed by using the NTSYS-pc computer package (Rohlf, 1992) and SAS (SAS Institute, 1988).

## Results

### DNA variation revealed by RFLPs

From the 104 CECs used in this study, 90 (85%) revealed polymorphic RFLP patterns and remaining 14 monomorphic across the 105 inbreds. When the 58 R- and 47 B-lines were analyzed separately, 89 CECs showed polymorphic RFLP patterns in the former group and 82 in the latter. The maximum number of patterns detected by a single CEC was 13 for R-lines and 11 for B-lines.

A total of 326 different RFLP patterns was present across the entire set of inbreds for the 104 CECs. Two hundred and seventy six were common to R- and B-lines, 32 were only found in R-lines, and 18 only in B-lines. For the R-lines, there were 39 CECs revealing single-band patterns and 65 revealing multiple-band patterns. In the B-lines, 54 CECs revealed single-band and 50 revealed multiple-band patterns. The distributions of the number of patterns per CEC for R- and B-lines are shown in Fig. 1. An average of 3.6 patterns per CEC was detected among R-lines but this value dropped to 3.0 in the B-lines. Although there were more R- than B-lines, these results suggest more variation within the R-lines.

### Relatedness as measured by *GS* and *f*

Histograms showing the distribution of *GS* and *f* estimates for R- and B-line combinations are presented in Fig. 2. *GS* estimates for R-lines ranged from 0.51 to 0.96 and averaged 0.67 (Fig. 2A). B-lines presented a greater average *GS* (0.76) than the R-lines but a similar range of 0.51 to 0.98 (Fig. 2B).

Estimates of  $f$  were low in both groups of lines. R-lines had a greater average (0.08) than B-lines (0.07) with a common range of 0 to 0.62 (Fig. 2C, 2D). These results from pedigree records differed from RFLP analyses which suggest a higher level of genetic similarity among B-lines.

#### **Multivariate analysis of RFLP data**

Associations among the 105 inbred lines obtained from UPGMA cluster analysis of GS estimates are illustrated in Fig. 3. R- and B-lines diverged at about  $GS = 0.65$ , forming distinct groups in close accordance with parental type, pedigree, and the classification system utilized by breeders. There were some obvious exceptions, R-lines 011 and 045 were placed into the B- group, and B-line 105 was placed into the R- group. Also, lines 033 (R-), 065 (B-), and 101 (B-) with  $GS \leq 0.65$  clustered apart from the other lines.

The R-lines were grouped in two main clusters (I and II) on the dendrogram. Cluster I was composed of three main sub-clusters containing lines with the following pedigree: sub-cluster I -1 with one line Fe4Ze4 (25% Feterita, 25% Zera-zera) and one Ka3 (50% Kafir); sub-cluster I - 2 with five lines Fe1 (100% Feterita), two Fe3 (50% Feterita), one Fe3Gi3 (Feterita x Giza), one Ze3 ( 50% Zera-zera), and one No3Gr4 (50% Norghum, 25% Graingrass); and sub-cluster I - 3 with two groups of lines, one consisting of two Ze3, one Fe3, three No3, one Gr1 (100% Graingrass), and one Fe3Ze4, and the other group of one Fe3, one Fe3Ze4, one Gr3Ze4 (50% Graingrass , 25% Zera-zera), one Ze3Fe4, one Ze3, and one Fe4.

Cluster II was also composed of three main sub-clusters containing lines with the following pedigree: sub-cluster II - 1 with two Ze3, one Ze1, and one Ye3Ze3 (Yellow x Zera-zera); sub-cluster II - 2 with two groups of lines, one of them consisting of one Gr3, one No3, and one complex source, and the other group of three Ye3Fe3, one Ze2 (75% Zera-zera), one Fe3Ze3, and one Ze4Su4 (25% Zera-zera, 25% Sudangrass); sub-cluster II - 3 with two groups of lines, one of them consisting of four Ze1, six Ze3, one Ze4, one Ze3Fe3, one Fe3, and the other group of two Ze3Fe5 (50%Zera-zera, 12.5% Feterita), one Ze3, and one Ze4.

The B-lines were grouped in different sub-clusters. In sub-cluster III - 1 and III - 2 clustered lines with high level of GS. Lines derived from Redlan, Martin and Wheatland clustered in sub-cluster III - 1 while in sub-cluster III - 2 grouped some lines derived from Kafir. In the remaining sub-clusters, lines were not clearly separated according to pedigree and germplasm sources.

Principal component analysis (PCA) for the R- and B-lines' RFLP data showed similar results to cluster analysis. The first (PC1), second (PC2) and the third (PC3) principal components accounted for 8.8%, 6.3% and 5.6%, respectively, for R-line variation, and 11.6%, 7.2% and 6.2%, respectively, for B-line variation. The R-lines were spread across the plot forming three main loose groupings (Fig. 4). Feterita germplasm formed a cluster with a few other lines either from the same source or from early introduced sources at one extreme of the plot with positive values of PC1 and PC3. Lines derived from Zera-zera formed an other cluster with



positive values of PC3 but negative values of PC1. The third group, in the center of the plot was composed of lines derived from hybrids between Zera-zera and Feterita and a mixture of the remaining germplasm sources, partially overlapping the two extreme clusters.

Most of the B-lines formed one tight cluster in one extreme of the plot with positive values for PC1 and values near zero for PC2 (Fig. 5). Germplasm from groups Kafir, Zera-zera, Martin, Redlan, Wheatland, Plainsman, and Yellow were clustered in the same group. The exceptions were lines 105 (Kafir), 101 (Yellow), 065 (Complex population), 076 (Kafir), and 086 (Kafir).

The regression of  $GS$  on  $f$  examines the relationship between these two similarity estimates (Fig. 6 and 7). There was a significant ( $P < 0.01$ ) moderate to low correlation for 825 related ( $f > 0$ ) pairs of R-lines ( $r = 0.46$ ) and for 549 related pairs of B-lines ( $r = 0.43$ ). Although these correlations are not high, they may suggest that RFLP data is a potential tool for quantifying the degree of pedigree relatedness in elite sorghum germplasm.

### Discussion

The sorghum industry in U.S. exploits a limited portion of the available genetic diversity. Important limiting factors include the amount of time required to convert the tropical varieties into short, day-neutral genotypes and the almost exclusive use of the Kafir x Milo cytoplasmic-genetic male-sterility system for the production of hybrid seeds (Miller, 1979; Duncan et al., 1991). Differences in

breeding approaches to derive sorghum R- and B-lines led us to infer that R-lines would have a higher level of genetic diversity.

#### **Variation at the DNA level**

Even though there were more R- than B-lines in this study, the differences at the DNA level revealed by the RFLP data showed more variation in the former group of lines. In the R- group of lines, there were more CECs revealing polymorphic patterns, higher average of patterns per CEC, and greater numbers of multiple and unique bands (bands only found in one group of lines). The level of polymorphism reported here is higher than in previous studies of sorghum (Whitkus et al., 1992; Tao et al., 1993; Vierling et al., 1994) or in other autogamous crops, such as rice (*Oryza sativa* L.; Wang and Tanksley, 1989) and barley (Melchinger et al., 1994). This could be due to the greater number of mapped markers and lines investigated. Unique RFLP bands present in the R- and B- inbreds were distributed among lines. However, lines in the R- group with Zera-zera germplasm in their pedigree and in the B- group with Kafir showed higher frequencies of unique bands. Differences at the DNA level between R- and B- group of lines may be maintained, not only at the RFLP loci investigated, but all across the genome. This genetic divergence could be associated with the heterosis manifested in hybrids between these two germplasm pools. CECs yielding multiple-band RFLP patterns contributed twice that of CECs generating single-band patterns to the observed values of patterns per probe in both group of lines. Multiple-band RFLP patterns may be due to binding

sequence repetition in the genome (Helentjaris et al., 1988) or to different levels of heterogeneity and heterozygosity of the lines. The difference in the number of multiple-band patterns found in R- and B-lines appears to be due to the latter cause. B-lines should have higher levels of homogeneity and homozygosity because of the breeding procedures (Miller, 1979) used to develop and maintain these lines.

### **Genetic similarity**

Our findings support the sorghum breeder's hypothesis of less genetic diversity within A-B- than R-lines. The average GS estimates were significantly higher in B- than R-lines (Fig. 2A, B). On average, any two B-lines had 76% of the RFLP bands in common, though in the R-lines, this value dropped to 67%. Reduced variability among B-lines might be due to the wide use of the unique genetic male-sterility system (Kafir nuclear factors and Milo cytoplasm) for the production of these lines. Most B-lines in this study either have the Milo-Kafir male sterility factors or were selected from segregating populations originating between crosses of lines from these germplasm sources.

The production of A-B-lines is accomplished by backcross breeding as discussed by Miller (1979). It has been shown that during backcross procedure a certain amount of introgressed segments originated from the donor parent are left in the backcrossed derived genotype (Young and Tanksley, 1989). The introgressed segments and the unique cytoplasm (Milo) used for the production of A-B-lines might be the ultimate cause for narrowness among these parental lines.

The level of genetic diversity detected in R- and B-lines in our study was greater than that obtained in sorghum by using RAPDs (Tao et al., 1993) and similar to earlier results from RFLPs (Vierling et al., 1994). However, diversity was less than that reported in maize inbred lines (Smith et al., 1990; Melchinger et al., 1991; Boppenmaier et al., 1992). The observed levels of genetic diversity may result from the sources of germplasm historically incorporated into sorghum breeding programs for the production of superior cultivars. According to Duncan et al. (1991), the sorghum genetic base has been widened through the utilization of converted materials released by the Sorghum Conversion Program.

Similarity estimates defined by  $f$  were low in both sets of lines (Fig. 2C, D). Values of  $f$  for many pairs of lines were zero (0) and this contributed to a decrease in the overall average. The  $f$  estimates are not in agreement with previous reports in sorghum, which suggest a higher level of genetic similarity among B-lines (Poehlman, 1987; Duncan et al., 1991). These findings could be due to either nonexistence of a detailed pedigree record in sorghum or the use of several simplifying assumptions (see Material and methods) to calculate  $f$  that may not be entirely accurate for elite sorghum inbred lines.

### **Multivariate analyses**

Cluster analysis of GS estimates for the 105 lines revealed overall associations among inbreds, showing groupings in accordance with parental types, pedigree records, and the classification system utilized by breeders (Fig. 3). With a

few exceptions, R- and B-lines were separated into different groups indicating that their differences at the DNA level could be assessed by the RFLP procedure used in this study. R-lines were split in two main clusters (I and II) as indicated in the dendrogram. Cluster I contains mostly lines derived from early introductions, mainly from Feterita germplasm. Feterita is commonly found in the parentage of hybrids and was introduced in U.S. in the early stage of sorghum development (around 1907) from the Sudan region of Africa (Poehlman, 1987). Lines derived 100% from Feterita diverged at high levels of GS and were grouped with some other lines in sub-cluster I - 2. Their high level of similarity indicate that they were probably derived from a narrow genetic base. Lines derived from Norghum and Graingrass were grouped with lines derived from Zera-zera and Feterita in sub-cluster I - 3. In this sub-cluster there was also another group of lines including mainly hybrids between the last two germplasm groups. Most of these lines have incomplete pedigree data and may share some common germplasm with others from cluster I. Cluster II contains mostly lines derived from recent introductions, mainly Zera-zera and Yellow germplasm. Zera-zera, widely used as R- parental types, was introduced from Ethiopia and became known to breeders in the 1970's as a source of genes for resistance to downy mildew and high yielding (Kramer, 1987). Most of the lines derived 50 and 100% from Zera-zera were grouped in sub-clusters II - 1 and II - 3, and sub-cluster II - 2 grouped lines derived from hybrids of Zera-zera and Yellow and from other sources. Lines derived from more recent introductions, in

contrast to lines derived from early introductions, are more clearly classified by source.

Most B-lines diverged at higher levels of GS and were grouped in different sub-clusters. Lines derived from Martin, Redlan, Wheatland, Plainsman (sub-cluster III -1) and, a few from Kafir (sub-cluster III - 2) showed high levels of similarity. These results agree with published reports that consider Plainsman, Martin, Redlan and Wheatland to be derived from crosses of Kafir and Milo (Poehlman, 1987; Kramer, 1987; Duncan et al., 1991). Lines derived from these germplasm sources should be highly related to each other and to their progenitor, Kafir. The remaining lines, mostly with unknown complete pedigree, derived from Kafir (and a few from Feterita, Zera-zera, and Yellow) germplasm diverged at different levels of GS and were not clearly classified according to pedigree and germplasm source.

The associations among germplasm groups revealed by PCA agreed with the results obtained by cluster analysis. The variation explained by the first three PCS were low for R- (20.7%) and B- (25%) lines. The first PC apparently explains the variation among germplasm sources, and the second and third may explain the variation within sources. The R- lines were widely spread all over the plot, forming three main loose clusters (Fig. 4). Feterita and Zera-zera derived lines were each grouped in extremities of the plot, indicating divergence between these two germplasm groups. In contrast, most of the B-lines formed one tight cluster (Fig. 5). Kafir, Redlan, Martin, Wheatland, Plainsman, Feterita, and Zera-zera derived lines

were grouped in one tight cluster. This grouping may be caused by the common germplasm introgressed from Kafir and Milo sources during transformation of these lines into A-B- parental lines.

Finally, the results obtained by these multivariate procedures agree with what is known about the breeding history of these lines and provide new insights about the relationships of the different germplasm sources used in the sorghum industry.

#### **Relationship between GS and $f$**

Genetic similarity based on RFLPs and  $f$  can be used as alternative measures of genetic relationships among genotypes. RFLP-derived GS estimates measure the proportion of bands in common between two genotypes. On the other hand,  $f$  estimates calculated indirectly from pedigree data by probability theory and Mendelian laws measure the proportion of alleles identical by descent that are shared between two genotypes (Falconer, 1988).

Although obtained by different approaches, GS and  $f$  showed a significant ( $P < 0.01$ ) moderate to low correlation for related ( $f > 0$ ) pairs of R- ( $r = 0.46$ ) and B- ( $r = 0.43$ ) lines (Fig. 6 and 7). The graphs also show that, in general, pairs of lines with low  $f$  values were widely spread about the correlation line, not correlating very well with GS. In contrast, lines with higher  $f$  values even though dispersed around the line were more correlated with GS. Correlations were lower than that ( $r = 0.93$ ) obtained in maize (Smith et al., 1991). In that study, however, many of the inbreds were related by pedigree, and a great abundance of pedigree data existed reflecting

the longer history of pedigree breeding in maize compared to sorghum. Finally, these results may indicate that RFLP data are applicable for quantifying the degree of pedigree relatedness in elite sorghum inbred lines.

### References

- Aldrich, P.R., and Doebley, J. 1992. Restriction fragment variation in the nuclear and chloroplast genomes of cultivated and wild *Sorghum bicolor*. *Theor. Appl. Genet.* 85:293-302.
- Aldrich, P.R., Doebley, J., Schertz, K. F., and Stec, A. 1992. Patterns of allozyme variation in cultivated and wild *Sorghum bicolor*. *Theor. Appl. Genet.* 85:451-460.
- Berhan, A.M., Hulbert, S.H., Butler, L.G., and Bennetzen, J.L. 1993. Structure and evolution of the genomes of *Sorghum bicolor* and *Zea mays*. *Theor. Appl. Genet.* 86: 598-604.
- Boppenmaier, J., Melchinger, A.E., Brunklaus-Jung, E., Geiger, H.H., and Herman, R.G. 1992. Genetic diversity for RFLPs in European maize Inbreds: I. Relation to performance of flint x dent crosses for forage traits. *Crop Sci.* 32:895-902.
- Doggett, H. 1988. *Sorghum*. Longmans, Green & Co., London.
- Duncan, R.R., Bramel-Cox, P.J., and Miller, F.R. 1991. Contributions of introduced sorghum germplasm to hybrid development in the USA. *CSSA special publication No. 17*. pp. 69-101.
- Helentjaris, T., Weber, D., and Wright, S. 1988. Identification of the genomic locations of duplicated nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. *Genetics* 118:353-363.
- Hulbert, S.H., Richter, T.E., Axtell, J.D., and Bennetzen, J.L. 1990. Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. *Proc. Natl. Acad. Sci. (USA)*. 87: 4251-4255.
- Falconer, D.S. 1989. *Introduction to quantitative genetics*. 3rd ed. John Wiley & Sons, Inc., NY.



- Kramer, N.W. 1987. Grain sorghum production and breeding Historical perspectives to future prospects. *Proc. Annu. Corn. and Sorghum Res. Conf.* 42:1-19.
- Lee, M., Godshalk, E.B., Lamkey, K.R., and Woodman, W.W. 1989. Association of restriction fragment length polymorphisms among maize inbreds with agronomic performance of their crosses. *Crop Sci.* 29:1067-1071.
- Melchinger, A.E., Messmer, M.M., Lee, M., Woodman, W.L., and Lamkey, K.R. 1991. *Diversity and relationships among U.S. maize inbreds revealed by restriction fragment length polymorphisms.* *Crop Sci.* 31:669-678.
- Melchinger, A.E., Graner, A., Singh, M., and Messmer, M.M. 1994. Relationships among European barley germplasm: I. Genetic diversity among winter and spring cultivars revealed by RFLPs. *Crop Sci.* 34:1191-1199.
- Miller, F.R. 1979. The breeding of sorghum. p. 128-136. In M. K. Harris (ed) *Biology and breeding for resistance to arthropods & pathogens in agricultural plants.* Texas A & M Univ., Texas Agric. Exp. Stn. MP-1451.
- Morden, C. W., J. Doebley, and K. F. Schertz. 1990. Allozyme variation among the spontaneous species of *Sorghum* section *Sorghum* (Poaceae). *Theor. Appl. Genet.* 80: 296-304.
- Mumm, R.H., and Dudley, J.W. 1994. A classification of 148 U.S. maize inbreds: I. Cluster analysis based on RFLPs. *Crop Sci.* 34:842-851.
- Nei, M., and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. (USA)* 76:5369-5273.
- Pereira, M.G., Lee, M., Bramel-Cox, P., Woodman, W.L., Doebley, J., and Whitkus, R. 1994. Construction of an RFLP map in sorghum and comparative mapping in maize. *Genome* 37: 236-243.
- Poehlman, J.M. 1987. Breeding sorghum and millet. p. 508-558. In *Breeding field crops.* AVI Pub Co., Westport, Connecticut.
- Rohlf, F.J. 1993. NTSYS-pc numerical taxonomy and multivariate analysis system. Version 1.80. Applied Biostatistics Inc., Setauket, NY.

- SAS Institute. 1988. SAS introductory guide for personal computers. SAS Institute, Inc., Cary, North Carolina.
- Smith, O.S., Smith, J.S.C., Bowen, S.L., Tenborg, R.A., and Wall, S.J. 1990. Similarities among a group of elite maize inbreds as measured by pedigree,  $F_1$  grain yield, heterosis, and RFLPs. *Theor. Appl. Genet.* 80:833-840.
- Smith, J.S.C., Smith, O.S., Bowen, S.L., Tenborg, R.A., and Wall, S.J. 1991. The description and assessment of distances between inbred lines of maize. III. A revised scheme for the testing of distinctiveness between inbred lines utilizing DNA RFLPs. *Maydica* 36: 213-226.
- Steel, R.G.D., and Torrie, J.H. 1980. Principles and procedures of statistics, A biometrical approach. 2nd ed. McGraw-Hill Book Co., New York.
- Tao, Y., Manners, J.M., Ludlow, M.M., and Henzel, R.G. 1993. DNA polymorphisms in grain sorghum (*Sorghum bicolor* (L.) Moench). *Theor. Appl. Genet.* 86: 679-688.
- Veldboom, L.R., Lee, M., and Woodman, W.L. 1994. Molecular marker-facilitating studies in an elite maize population: i. Linkage analysis and determination of QTL for morphological traits. *Theor. Appl. Genet.* 88: 7-16.
- Vierling, R.A., Xiang, Z., Joshi, C.P., Gilbert, M.L., and Nguyen, H.T. 1994. Genetic diversity among elite sorghum lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs. *Theor. Appl. Genet.* 87:816-820.
- Wang, Z.Y., and Tanksley, S.D. 1989. Restriction fragment length polymorphism in *Oryza sativa* L. *Genome* 32:1113-1118.
- Whitkus, R., Doebley, J., and Lee, M. 1992. Comparative genome mapping of sorghum and maize. *Genetics* 132: 1119-1130.
- Young, N.D., and Tanksley, S.D. 1989. RFLP analysis of size of chromosomal segments retained around the Tm-2 locus of tomato during backcross breeding. *Theor. Appl. Genet.* 77: 353-359

Table 1. Pedigree background of the 105 elite sorghum inbred lines used for RFLP data collection.

Line†	Pedigree††	Abbr.†††	Line†	Pedigree††	Abbr.†††
<b>R-lines</b>					
001	50% Norghum	No3	055	Yellow x Feterita	Ye3Fe3
002	100% Zera-zera	Ze1	056	50% Zera-zera; 12.5% Feterita	Ze3Fe5
003	100% Feterita	Fe1	057	50% Zera-zera; 12.5% Feterita	Ze3Fe5
004	50% Norghum	No3	058	50% Zera-zera	Ze3
005	Feterita x Giza	Fe3Gi3			
006	50% Feterita	Fe3			
007	50% Feterita	Fe3	<b>B-lines</b>		
008	50% Norghum	No3	059	50% Kafir	Ka3
009	50% Zera-zera	Ze3	060	25% Kafir	Ka4
010	100% Zera-zera	Ze1	061	# Martin; Kaoling	MaKo
011	50% Feterita	Fe3	062	# Combine Kafir	CK
012	50% Graingrass	Gr3	063	# Martin; Redlan	MaRe
013	50% Feterita	Fe3	064	100% Kafir	Ka1
014	50% Feterita	Fe3	065	# Complex population	Cp
015	50% Zera-zera	Ze3	066	25% Kafir	Ka4
016	50% Zera-zera	Ze3	067	100% Zera-zera	Ze1
017	25% Feterita	Fe4	068	25% Kafir	Ka4
018	100% Feterita	Fe1	069	# Complex population	Cp
019	50% Graingrass; 25% Zera-zera	Gr3Ze4	070	25% Kafir	Ka4
020	50% Norghum	No3	071	25% Kafir	Ka4
021	50% Zera-zera	Ze3	072	25% Kafir	Ka4
022	50% Feterita; 25% Zera-zera	Fe3Ze4	073	100% Zera-zera	Ze1
023	100% Graingrass	Gr1	074	Kafir x Part converted	Ka3Pa3
024	50% Zera-zera	Ze3	075	100% Kafir	Ka1
025¶	100% Zera-zera	Ze1	076	100% Kafir	Ka1
026	Yellow x Feterita	Ye3Fe3	077	25% Kafir	Ka4
027	Zera-zera x Feterita	Ze3Fe3	078	25% Kafir	Ka4

† Line names are not publicly available. †† Pedigree information provided by Pioneer Hi-bred International. ††† Pedigree abbreviations.

¶ Forage sorghum, all the others are grain sorghum. # Percentage of germplasm for each parent is unknown.

Table 1. (Continued)

Lin <sup>et</sup>	Pedigree <sup>tt</sup>	Abbre. <sup>ttt</sup>	Lin <sup>et</sup>	Pedigree <sup>tt</sup>	Abbre. <sup>ttt</sup>
<b>R-lines</b>			<b>B-lines</b>		
028	50% Zera-zera	Ze3	079	25% Kafir	Ka4
029	50% Zera-zera	Ze3	080	# Martin; Kafir	MaKa
030	50% Feterita	Fe3	081	# Martin; Kafir	MaKa
031	75% Zera-zera	Ze2	082	# Martin; Kafir	MaKa
032	50% Zera-zera; 25% Feterita	Ze3Fe4	083	50% Martin; 25% Redlan; 25% Kaoling	Ma3Re4
033	# Complex population	Cp	084	50% Wheatland	Wh3
034	50% Zera-zera	Ze3	085	# Martin; Kafir	MaKa
035	50% Zera-zera	Ze3	086	50% Kafir	Ka3
036	50% Zera-zera	Ze3	087	25% Kafir	Ka4
037	100% Feterita	Fe1	088	50% Plainsman; 25% Martin	Pl3Ma4
038	100% Feterita	Fe1	089	100% Martin	Ma1
039 <sup>¶</sup>	25% Zera-zera; 25% Sudangrass	Ze4Su4	090	Plainsman x Martin	Pl3Ma3
040	Feterita x Zera-zera	Fe3Ze3	091	100% Yellow	Ye1
041	# Complex population	Cp	092	100% Redlan	Re1
042	Yellow x Zera-zera	Ye3Ze3	093	100% Redlan	Re1
043	50% Norghum; 25% Graingrass	No3Gr4	094	# Kafir x Part converted	KaPa
044	50% Feterita; 25% Zera-zera	Fe3Ze4	095	50% Martin	Ma3
045	100% Yellow	Ye1	096	25% Kafir	Ka4
046	100% Feterita	Fe1	097	50% Kafir	Ka3
047	50% Zera-zera	Ze3	098	# Kafir; Complex population	KaCp
048	25% Zera-zera	Ze4	099	25% Kafir	Ka4
049	50% Zera-zera	Ze3	100	25% Kafir	Ka4
050	100% Zera-zera	Ze1	101	100% Yellow	Ye1
051	25% Feterita; 25% Zera-zera	Fe4Ze4	102	25% Kafir	Ka4
052	Yellow x Feterita	Ye3Fe3	103	25% Kafir	Ka4
053	100% Zera-zera	Ze1	104	50% Kafir	Ka3
054	25% Zera-zera	Ze4	105	50% Kafir	Ka3

Table 2. Linkage group location of probes used for RFLP analysis of the 105 sorghum inbred lines.

Linkage group†	Probe designation	Number of probes
A	ISU074, ISU087, ISU161, ISU096, ISU106, UMC023	6
B	ISU058, ISU076, ISU086, ISU096, ISU101, ISU138, ISU142, ISU144, NPI327	9
C	BNL05.09, BNL08.17, BNL12.06, ISU027, ISU041, ISU049, ISU05, ISU061, ISU070, ISU081, ISU082, ISU162, ISU122, UMC076	14
D	BNL05.71, ISU035, ISU042, ISU047, ISU056, PIO20-566, PIO20-598, PIO20-608, UMC108	9
E	ISU140, UMC064	2
F	BNL06.20, BNL14.07, BNL15.21, ISU037, ISU043, ISU050, ISU066, PIO15-37, PIO20-728, UMC088, UMC122, UMC136	12
G	BNL05.37, BNL06.16, BNL08.35, ISU039, ISU052, ISU114, PIO20-726, UMC0.93	8
H	BNL03.06, PIO10-5, UMC113, UMC114	4
I	ISU036, NPI379	2
J	ISU055, ISU059, ISU060, ISU065, ISU109, ISU120, ISU137, UMC008	8
¶	BNL05.67, BNL08.04, BNL09.08, BNL09.11, ISU031, ISU038, ISU040, ISU046, ISU155, ISU157, ISU158, ISU159, ISU160, ISU163, ISU164, ISU124, ISU165, ISU166, UMC005, UMC010, UMC011, UMC034, UMC046, UMC083, UMC090, UMC109, UMC167, UMC119, UMC134, UMC168	30
		104

† Linkage group from, Pereira et al., 1994.

¶ Probes not mapped in sorghum.

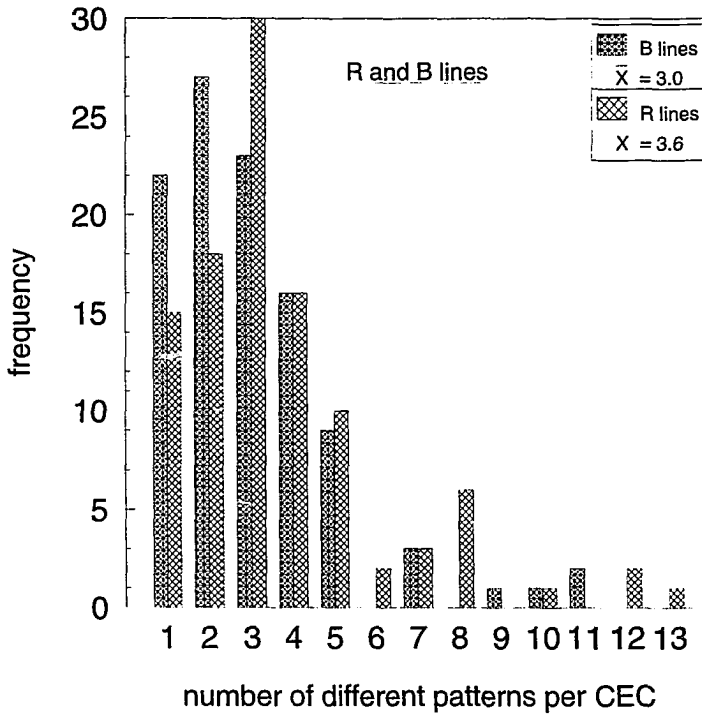


Figure 1. Frequency distribution of the number of RFLP patterns revealed by 104 clone-enzyme combinations (CECs) among 58 R- and 47 B-lines.  $\bar{X}$  indicates the average number of patterns per CEC.

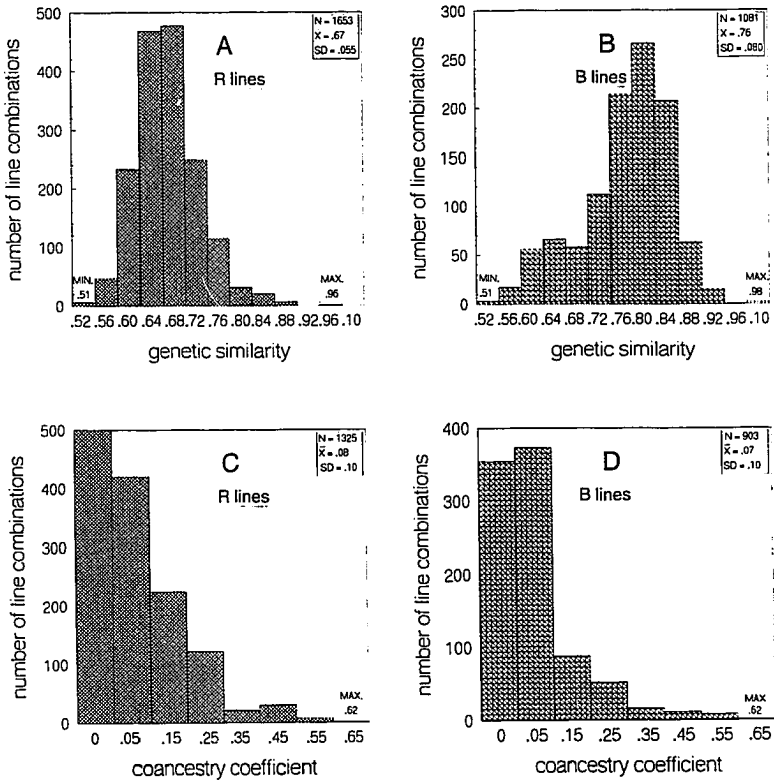


Figure 2. Histograms of genetic similarity (GS) and coancestry coefficient ( $f$ ) estimates, obtained from RFLP and pedigree data, respectively. Panel A refers to GS of R-lines and Panel B to GS of B-lines. Panel C refers to  $f$  of R-lines and Panel D to  $f$  of B-lines. N indicates the number of line combinations in each group,  $\bar{X}$  the average, and SD the standard deviation.

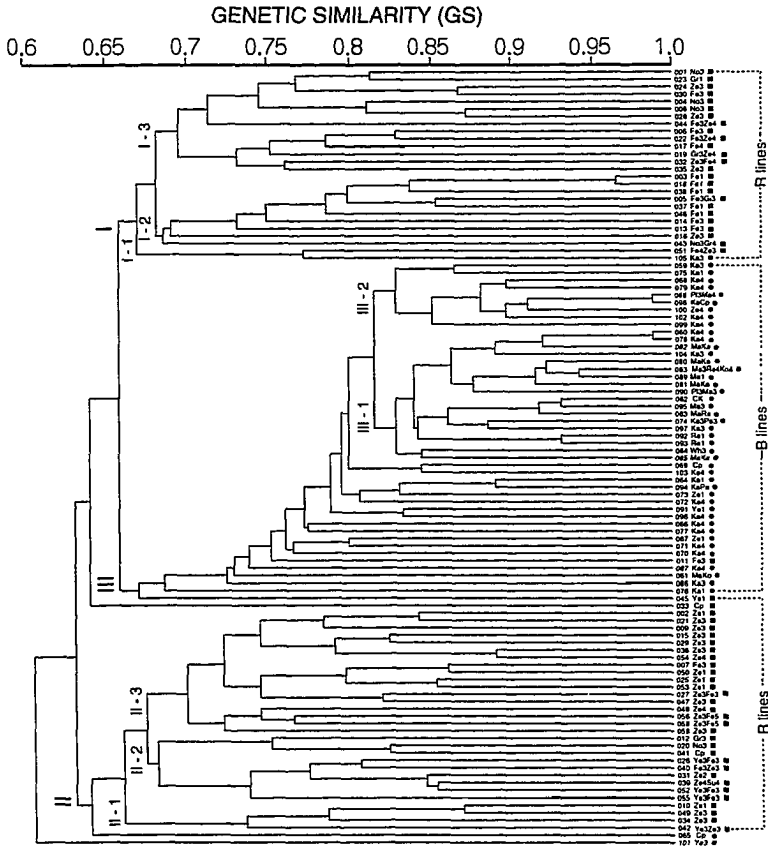


Figure 3. Dendrogram of the 105 sorghum inbred lines revealed by UPGMA cluster analysis of RFLP-based genetic similarity (GS) estimates. (■) refers to R-lines and (●) to B-lines.



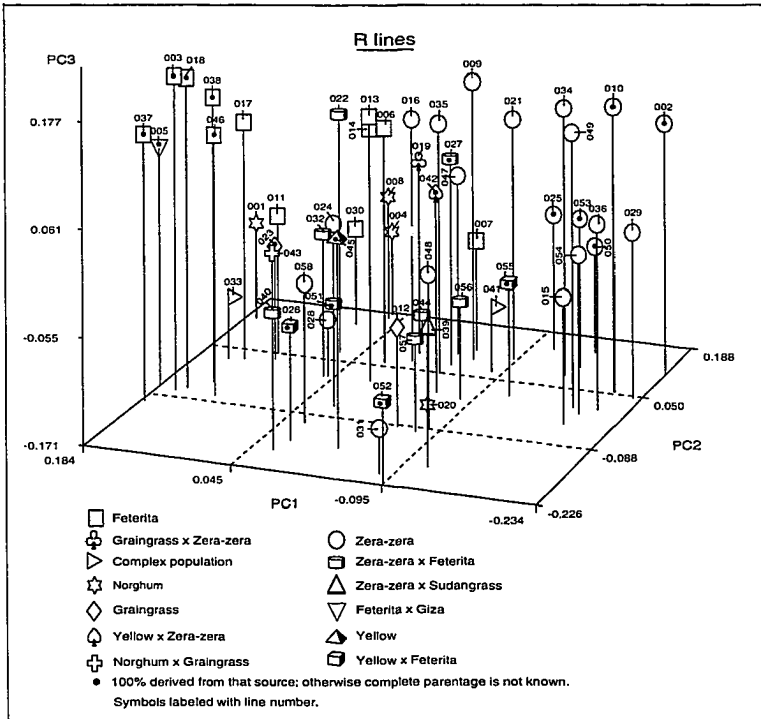


Figure 4. Associations among sorghum R-lines revealed by principal component analysis (PCA) performed on correlation matrices calculated from the RFLP data of 104 clone-enzyme combinations. PC1, PC2, and PC3 refers to 1st, 2nd, and 3rd principal coordinates.

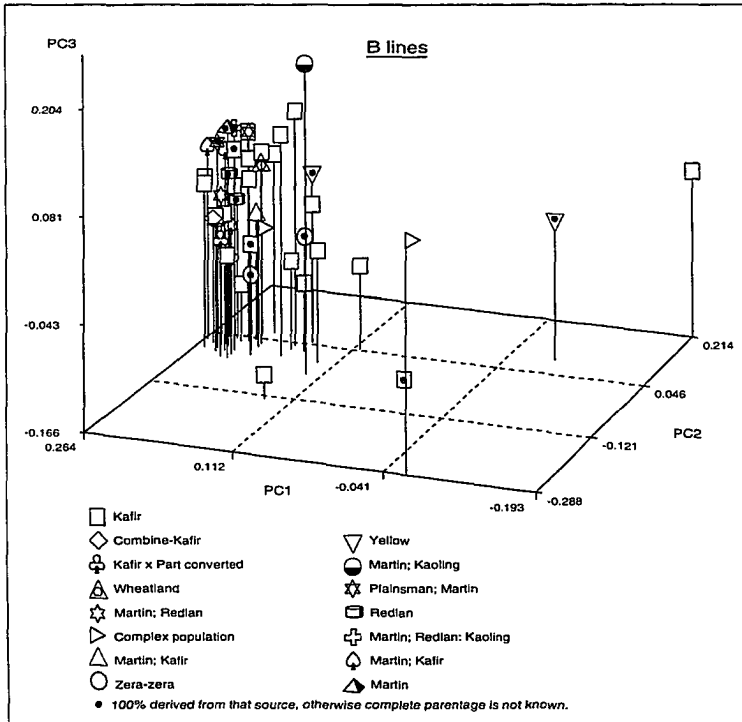


Figure 5. Associations among sorghum B-lines revealed by principal component analysis (PCA) performed on correlation matrices calculated from the RFLP data of 104 clone-enzyme combinations. PC1, PC2, and PC3 refers to 1st, 2nd, and 3rd principal coordinates.

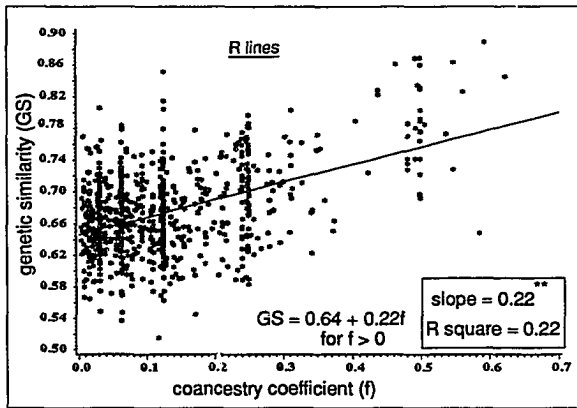


Figure 6. Scatter plot of genetic similarity (GS) versus coancestry coefficient ( $f$ ) estimates for related ( $f > 0$ ) pairs of sorghum R-lines.

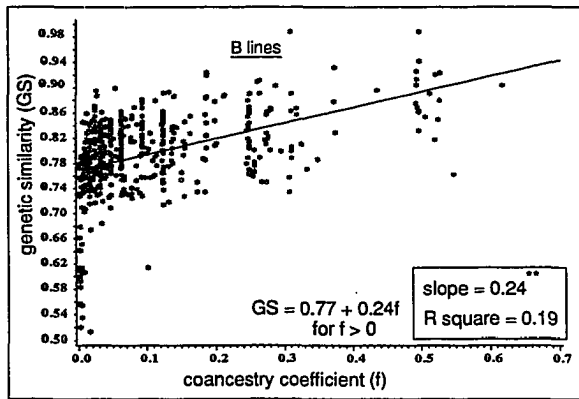


Figure 7. Scatter plot of genetic similarity (GS) versus coancestry coefficient ( $f$ ) estimates for related ( $f > 0$ ) pairs of sorghum B-lines.

## IDENTIFICATION OF PLANT HEIGHT QTL IN SORGHUM ACROSS GENERATIONS AND COMPARATIVE MAPPING WITH MAIZE

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### Abstract

Restriction fragment length polymorphisms (RFLPs) were employed to investigate quantitative trait loci for plant height on 152  $F_{2,3}$  sorghum [*Sorghum bicolor* (L.) Moench] lines. These lines were obtained from the cross CK60 (*Sorghum bicolor* ssp. *bicolor*) and PI229828 (*Sorghum bicolor* ssp. *drummondii*) and grown in a replicated trial. Our objectives were i) detect and characterize QTL for plant height with replicated progeny, ii) characterize QTL for other traits associated with regions of the plant height QTL, iii) compare estimates of QTL detected in  $F_2$  plants and their  $F_3$  progeny, and iv) provide additional evidence of orthologous regions for plant height and other traits between the genomes of maize and sorghum. Six QTL for plant height were located in the  $F_{2,3}$  and four in the  $F_2$  generations. The QTL mapped in the  $F_2$  coincided at the same regions in the  $F_{2,3}$  and had genetic effects of different magnitudes but in the same direction. As expected, the multiple QTL model explained larger variation in the  $F_{2,3}$  (77.5%) than in the  $F_2$  (63.4%). Plant height QTL were associated at the same region with QTL for other morphological traits suggesting linkage or pleiotropic effects.

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Plant height QTL were also identified to correspond with map location of qualitative loci *dw1*, *dw2*, *dw3*, and *dw4*, which control most of the variation for height. In comparing sorghum and maize linkage maps, four orthologous regions were identified affecting plant height and other traits in both species suggesting that these regions may have preserved their function during evolution.

### Introduction

Comparative genetic mapping of restriction fragment length polymorphisms (RFLPs) has revealed a high degree of conserved genetic linkage between the genomes of maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench] in several reports (Hulbert et al., 1990; Whitkus et al., 1992; Berhan et al., 1993; Pereira et al., 1994; Chittenden et al., 1994). The considerable synteny inferred on the basis of homologous, low copy DNA sequences suggests the possibility of conserved function (Fatokun et al., 1992; Ahn et al., 1993; Van Deynze et al., 1995). Detection of quantitative trait loci (QTL) for plant height in sorghum and comparative mapping with maize DNA probes have provided evidences for orthologous QTL for this trait and others in maize (Pereira and Lee, 1995; Lin et al., 1995). Herein, we provide additional data supporting those observations through analysis of replicated progenies and other traits associated with sorghum plant height QTL.

In sorghum, plant height has been genetically manipulated by breeders for the production of short stature F<sub>1</sub> hybrids adapted to mechanized harvesting

(Poehlman, 1987). The character has several components such as internode length, peduncle length, node number, and panicle length; however, most investigations have concentrated on internode length because it is the primary determinant of the trait (Doggett, 1988). Four unlinked genetic loci (*dw1*, *dw2*, *dw3*, and *dw4*) with major effects on internode length have been identified through qualitative (Quinby and Karper, 1954) and quantitative genetic analysis (Hadley, 1957). Both studies reported additional genetic factors influencing this trait. However, the relatively minor influence of those factors prevented further genetic characterization. Alleles with dominant effects at the *Dw* loci confer increased plant height primarily through the elongation of the internodes. In general, maximum height (ie. four meters) requires a dominant allele at each *dw* locus. Complete substitution of recessive for a dominant allele at any of the four loci may decrease the stature by 50 cm, while additional substitutions at the remaining loci typically result in less reductions in height (House, 1985). Pleiotropic or linkage effects have been attributed to some of the alleles at the *Dw* loci. Alleles at the *dw3* have been associated for variation of number of tillers, panicle size, number of seeds per panicle, and seed weight (Casady, 1965). Likewise, alleles at *dw2* have effects on panicle length, seed weight, grain yield at the main panicle, and leaf area (Graham and Lessman, 1966). In contrast, alleles at the *dw4* have not been reported to be associated with effects on traits other than plant height (Quinby, 1932; Quinby and Karper, 1954). To our knowledge, the *dw1* locus has not been investigated with

regard to effects on other traits. Also, the biochemical basis of reduced stature attributed to the *Dw* loci has not been established.

Recent genetic analysis of QTL for plant height in sorghum has detected and characterized several regions, some with characteristics resembling those of the *Dw* loci. Pereira and Lee (1995) reported four unlinked QTL for plant height with dominant alleles for increased height at three of the loci. Similar results have been reported by Lin et al. (1995) who detected six QTL in a larger and more divergent population. In both studies, trait data were collected directly from  $F_2$  plants. On the basis of genetic associations between the plant height QTL and QTL for other traits (reported herein) and the estimate of genetic effects of the plant height QTL, Pereira and Lee (1995) hypothesized that some plant height QTL may reflect allelic variation at *Dw* loci. This is consistent with the concept that some QTL and genetic loci are one in the same (Robertson 1985; Beavis et al., 1991; Doebley and Stec, 1993). On the basis of introgressed regions supposedly harboring *dw2* and maturity *ma1* loci and the estimate of genetic effects of QTL for plant height and flowering date, Lin et al. (1995) also associated QTL for plant height and flowering date with the *dw2* and maturity *ma1* loci, respectively.

In this study, we have used replicated  $F_3$  lines of the same population (Pereira and Lee, 1995) to confirm estimates of QTL for several traits reported for  $F_2$  plants. Specifically, the objectives of the study were i) detect and characterize QTL for plant height with replicated progeny, ii) characterize QTL for other traits



associated with regions of the plant height QTL, iii) compare estimates of QTL detected in  $F_2$  plants and their  $F_3$  progeny, and iv) provide additional evidence of orthologous regions for plant height and other traits between the genomes of maize and sorghum.

## **Materials and Methods**

### **Mapping progeny**

One hundred and fifty-two  $F_{2.3}$  lines were obtained by self-pollinating  $F_2$  plants from a cross between sorghum lines CK60 and PI229828. Self-pollination was assured by covering main panicles at the onset of anthesis. Selection of the mapping parents and their attributes have been described previously (Pereira and Lee, 1995). The inbred line CK60 (*Sorghum bicolor* spp. *bicolor*, race Kafir) has a short stature and is homozygous for the following alleles controlling plant height: *dw1*, *Dw2*, *dw3*, and *dw4* (Quinby and Karper, 1954). PI229828 is a tall weedy sorghum and belongs to *Sorghum bicolor* ssp. *drummondii* (Duncan et al., 1991; deWet, 1978). The allelic composition at the *Dw* loci of PI229828 has not been determined, but its phenotype suggests the presence of alleles of increased plant stature at each of the four loci.

### **Field design**

A total of 156 entries was evaluated in a 12 x 13 simple rectangular lattice design of one-row plots with two replications at the Agronomy and Agricultural Engineering Research Center near Ames, Iowa, in 1994. The plots were 3.05 m

long with 0.76 m spacing between rows. Around 50 seeds were hand-planted per plot on May 25, 1994. Plots were thinned to a uniform stand of 25 plants per plot. The entries consisted of 152  $F_{2,3}$  lines, the parents CK60 and PI229828, and the  $F_1$  planted twice in each replicate. Morphological traits were recorded on ten consecutive plants per plot and averaged to obtain an estimate on a plot mean basis. Data were recorded as follows:

Plant height (PH, the height of the plant measured at anthesis from the soil surface to the tip of the panicle);

Plant height to the uppermost node (PHUN, the height of the plant measured at anthesis from the soil surface to the uppermost node);

Panicle length (PAL, the length of the panicle measured at harvesting);

Number of nodes (NN; the number of nodes on the main stem counted at anthesis);

Peduncle length [PEL, the length of the peduncle obtained by the following difference:  $PH - (PAL + PHUN)$ ].

Panicle width (PAW, the width at the widest part of the panicle at harvesting);

Leaf length (LL, the length of the third leaf from the panicle at anthesis);

Leaf width (LW, the width of the widest part of the third leaf from the panicle at anthesis);

Number of tillers (TN, the number of tillers with seeds per plant counted at anthesis);

Stalk circumference (SCI, the circumference of the main stalk measured around 15 cm above the soil surface at anthesis);

Flowering date (GDD to anthesis) was recorded in accumulated growing degree days (GDD) in °C from planting to the date 50% of the plants in a plot reached anthesis. GDD were calculated from the daily minimum and maximum temperature as described by Hammer and Vanderlip (1989).

The F<sub>2</sub> generation was grown in 1992 and the following traits were recorded: PH, PAL, PAW, LL, LW, TN, SCI, anthesis, peduncle diameter (PED), number of seed-branches per panicle (NSB), seed-branch length (SBL), sterile portion of the seed-branch length (SSBL), and weight of 100 seeds in grams (SWT). Procedures for data collection in the F<sub>2</sub> plants were reported in Pereira and Lee (1995) and Pereira et al. (1995). Results from the F<sub>2</sub> will be used here for comparative purposes.

#### **RFLP assays and map construction**

RFLP assays and the procedures for map construction were previously described in Pereira and Lee (1995). RFLP patterns of locus *isu 147*, mapped on linkage group B, were rescored and the data used to recalculate distances between loci on this linkage group. The rescoring did not cause major changes in the RFLP linkage map. The map is composed of ten linkage groups defined by 111 loci, and has a total map distance of 1307 cM with an average distance between markers of 12.9 cM.

### **Data analysis**

Entry means of all traits were adjusted for lattice block effects and the effective error mean square calculated according to Cochran and Cox (1957). The adjusted plant height means of the 152 progenies were tested for normality by the W-test (Shapiro and Wilks, 1965). The parent-offspring regressions were calculated for all traits as described by Hallauer and Miranda (1988). Standard analysis of variances for a single rectangular lattice design model was performed. Sum of squares due to entry means were partitioned into sum of squares due to  $F_{2,3}$  progenies, checks, and progeny versus checks. The sum of squares due to checks was further partitioned into variation due to parents,  $F_1$ s, and  $F_1$ s versus parents.

Genotypic component of variance ( $\sigma^2_g$ ) estimates of the lines and the standard error (SE) were calculated as described by (Searle, 1971). Heritability was calculated on a progeny mean basis (Hallauer and Miranda, 1988). Exact 90% confidence interval on heritability estimates were calculated according to Knapp et al. (1985).

### **QTL identification**

QTL determinations were performed on the adjusted entry means using two different procedures. The first was interval mapping by using the computer program MAPMAKER/QTL version 1.1 (Lander and Botstein, 1989; Paterson et al., 1991). In these analyses, a LOD threshold of 2.4 was used based on Figure 4 of Lander and Botstein (1989). Initially, a single-QTL model was used to estimate individual

QTL map position. In this model, each QTL at a time was evaluated separately for each trait. Once individual QTL were defined, they were added to a multiple-QTL model (Lander and Botstein, 1989), and the total phenotypic variation and the additive and dominance effects were calculated (Jansen and Stam, 1994). Estimates of dominance effects were doubled as described in Mather and Jinks (1971) and Schön et al. (1993). Average levels of dominance were calculated by the ratio  $d/a$ , where  $d$  refers to the dominance effect in an  $F_2$  population, and  $a$  refers to the additive effect. Gene action was defined based on the average level of dominance according to the criteria proposed by Stuber et al. (1987): additive (A) = 0-0.20; partial dominance (PD) = 0.21-0.80; dominance (D) = 0.81-1.20; and overdominance (OD) > 1.20.

The second QTL detection procedure was single-factor analysis of variance (SFAOV) from the PROC GLM routine in SAS (SAS institute, 1988). Analyses were performed for each pair-wise combination of trait and RFLP locus (Edwards et al., 1987). F-tests at probability level ( $P \leq 0.01$ ) were conducted to verify if significant variation in trait expression was associated with differences in marker-locus genotypic classes. The presence of significant digenic epistasis among QTL identified for plant height was tested. Procedures for these analyses were previously described in Pereira and Lee (1995).

## Results

### Trait data analysis

The distribution of  $F_{2.3}$  line PH entry means significantly ( $P \leq 0.01$ ) deviated from normality ( $W = 0.92$ ; Shapiro and Wilk, 1965). Skewness toward taller genotypes was expected because height is partially dominant in sorghum (Quinby and Karper, 1954; Hadley, 1957). Similar distribution of PH was also observed in the  $F_2$  generation (Pereira and Lee, 1995). Although interval mapping assumes normal distribution of the trait data (Lander and Botstein, 1989), minor violation of this assumption should not alter QTL identification (Knott and Haley, 1992; Stuber et al., 1992; Veldboom et al., 1994; Beavis et al., 1994).

Plant height averages, variances, and heritabilities are summarized in Table

1. Genotypic variances of the  $F_{2.3}$  progenies ( $\delta^2g$ ) and their parents ( $\delta^2p$ ) were highly significant ( $P < 0.01$ ) indicating genetic differences for PH among these genotypes. Plant height in the  $F_{2.3}$  progenies averaged 286.8 cm and ranged from 114.6 to 353.8 cm. Averages of the parents and  $F_1$ 's were: 93.9 cm (CK), 312.0 cm (PI), and 298.4 cm ( $F_1$ ). Transgressive segregation was evident, as can be noticed by the higher averages of the  $F_{2.3}$  lines compared with the midparent value. Heterosis was observed in the  $F_1$  and  $F_{2.3}$  generations, which had higher PH averages than the midparent value. Broad sense heritability was high (90%), but this estimate may be biased upwards because it was obtained in just one environment. A complete statistical and biometrical analysis of the other traits will

be presented in a related manuscript.

#### **QTL identification**

Six unlinked regions on linkage groups A, B, D, E, F, and H were identified as being significantly associated with PH in this population (Table 2 ). Similar number of QTL for PH have also been reported in an interspecific  $F_2$  sorghum population (Lin et al., 1995). Except QTL on linkage group B, the regions were identified by both methods ( interval mapping and SFAOV) used in this study. The QTL on linkage group B located only by SFAOV had LOD score value of 2.2, close to the 2.4 levels used as the threshold. Individually, these QTL explained from 6.1 (linkage group B) to 30.2% (linkage group A) of the total phenotypic variation. The multiple QTL model accounted for 77.5% of the phenotypic variation in this population. As expected *a priori*, all six QTL had alleles derived from the tall parent (PI) resulted in increased stature. Partial to overdominance effects were observed for all QTL, except linkage group H. Thus, non-additive effects were important for the expression of height in this population.

Plant height averages at the nearest RFLP loci linked to QTL are summarized in Table 3. Progeny homozygous for CK alleles ranged from 248 cm on linkage group A to 275 cm on linkage group H. In contrast, progenies that were homozygous for PI alleles ranged from 289 (linkage group B) to 319 cm (linkage group H). The difference between these lines ranged from 21 to 51 cm on linkage group D and A, respectively. The magnitude of these differences indicates that

alleles at these QTL have large genetic effects.

Digenic epistatic interactions between two of the QTL for PH linked to marker loci *isu116* and *isu140* on linkage groups A and E, respectively, were highly significant ( $P \leq 0.01$ ; Table 4). The presence of PI alleles at marker locus *isu140* caused a reduction in the estimates of additive effects at QTL on linkage group A from 51 to 12 cm. Likewise, the presence of PI alleles at marker locus *isu116* also contributed to reduce the additive effects at QTL on linkage group E from 54 to 15 cm. Similar results were observed in the  $F_2$  population (Pereira and Lee, 1995).

#### **Comparison of plant height QTL in $F_2$ and $F_{2,3}$ generations**

The  $F_2$  and  $F_{2,3}$  generations were grown at the same location but in different years, 1992 and 1994, respectively. In those years, the weather conditions favored a good growing crop during the growing season. The estimated regression coefficient of the  $F_{2,3}$  progeny PH on the  $F_2$  parental PH was 0.70. This broad sense heritability estimate of PH in the  $F_2$  population (Hallauer and Miranda, 1988) reflected on the number of QTL in common across generations. All four PH QTL identified in the  $F_2$  generation were located at similar regions (on linkage groups A, B, E, and H) in the  $F_{2,3}$  (Figure 1). Placement of the QTL was virtually identical in both generations and environments. A very similar pattern of LOD score distributions along these linkage groups was observed for the QTL in common across generations. In general, QTL mapped in the  $F_{2,3}$  generation explained larger proportion of the phenotypic variation than QTL in the  $F_2$ . However, estimates of



additive effects were of similar magnitudes in the  $F_2$  and  $F_{2:3}$  generations. Partial to overdominance effects were the mode of gene action observed for most of the QTL in common. As expected, alleles from PI contributed to increased PH in all QTL in both generations. Also, the QTL on linkage group A showed the largest effects on PH in both generations. The two QTL detected only in the  $F_{2:3}$  generation on linkage groups D and F also had PI alleles associated with the additive effects, however, these effects were of smaller magnitudes than those observed for the QTL in common across generations.

#### **Association between QTL for plant height and other traits**

Plant height QTL were associated with QTL for other morphological traits in all linkage groups where they were located indicating linkage and/or pleiotropic effects (Figure 2). The position of QTL for height on linkage groups A, B, D, E, F, and H and 14 other traits were compared graphically. We considered a QTL for PH and any other trait be associated if their one-LOD score support intervals (SIs) defined by MAPMAKER-QTL overlapped or their peak LOD scores were less than 50 cM apart. Traits used for comparisons such as PAL, PAW, LL, LW, SCI, TN, and anthesis were measured in the  $F_2$  and  $F_{2:3}$  generations, PHUN, PEL, and NN only in the  $F_{2:3}$  and PED, NSD, SBL, and SSBL only in the  $F_2$ . Their QTL location, phenotypic effects, and directions of response on these linkage groups are described in Table 5.

All four QTL identified for the main height component PHUN overlapped with

SIs for PH on linkage groups A, E, F, and H. These QTL might be the main determinant of PH on these linkage groups and, QTL for PHUN also had PI alleles associated with increased trait value. Besides PHUN, twelve traits were associated with PH on linkage group A, eleven on linkage group H, and two on each linkage group E and F. Overlapping with SIs for PH QTL on linkage group A were PEL, NN, PAL, PAW, PED, NSB, SBL, SSBL, and SCI whereas TN, LW and anthesis were linked around 25 cM with PH but were not overlapping. In contrast to PH QTL, most of these QTL had CK alleles for increased trait value, even though they were mapped at the same region as PH. Identification of QTL with additive effects in opposite directions in the same region may indicate linked QTL. Overlapping with PH QTL on linkage group H and with the same parental effects were PEL, PAL, NN, PAW, LL, LW, SCI, anthesis, SBL, and SSBL, except PED and SWT. The only trait with CK alleles for increased value on this linkage group was SWT. Lin et al. (1995) also reported associations between QTL for PH and anthesis in sorghum, and attributed those associations to linkage rather pleiotropic effects. An important feature of linkage groups A and H is the high concentration of QTL with large effects in the  $F_2$ ,  $F_{2:3}$ , and in common across generations. These regions may have an important role in the expression of morphological traits in sorghum. Overlapping with SIs for PH QTL on linkage group E and in the same direction was PAL, and on linkage group F in opposite direction were LL and PAW. Stalk circumference was also associated with PH on linkage group E but in opposite direction. The

concentration of morphological traits in the same region may suggest the presence of a set (cluster) of QTL with linked and/or pleiotropic effects for all traits. Our findings seem to agree with the pleiotropic or linked effects attributed to some alleles at the dwarfing loci in sorghum. For example, the *dw2* and *dw3* loci besides affecting PHUN, have also effects on panicle components, leaf area, seed weight, grain yield, and tillering (Casady, 1965; Graham and Lessman, 1966).

An interesting feature of linkage groups B and D is the absence of QTL for the main height component PHUN associated with PH. Instead, were associated with PH components PAL and PEL on linkage group B, and PAL on linkage group D. Despite the importance of PHUN, these components might have contributed most of the variation for the identification of PH QTL on these linkage groups. Besides PAL, associated with PH QTL and in the same direction on linkage group B were LL, SCI, TN and PED, and on linkage group D PAW, TN, and LL. Leaf width was also associated with PH on linkage group B but with opposite parental effect. Tillering is an important agronomic trait in sorghum and was associated with PH on linkage groups A, B, and D. In all instances, QTL for TN and PH were not overlapping but linked by more than 25 cM. In contrast, most of the panicle components (PAL, PEL, PAW, SBL, SSBL, and NSBL) overlapped with PH QTL. Also, a QTL for flowering date with a large effect overlapped with the PH QTL on linkage group H. If desirable agronomic traits show pleiotropy or linkage, selection in one of these traits may result in correlated response in other traits. Therefore,

understanding the underlined genetic basis of the associations among morphological/agronomic traits could be of interest to sorghum breeding.

### Discussion

The larger number of QTL for PH identified in the  $F_{2.3}$  than  $F_2$  generations agreed with the increased power in QTL detection proposed for replicated progenies (Cowen, 1988; Soller and Beckman, 1990). A relatively large proportion of the total phenotypic variation for PH was accounted for by the multiple model including all QTL. As expected, the explained variation was higher in the  $F_{2.3}$  generation (77.5%) than in the  $F_2$  (63.4%). Nevertheless, this estimate was smaller than the heritability for height ( $h^2 = 90\%$ ) in the  $F_{2.3}$  generation. The heritability value is the upper limit of the phenotypic variation explained by QTL (Edwards et al., 1987). Therefore, a relatively small proportion of the variation (14%) was not accounted for by QTL in this study. The sum of phenotypic variation explained by individual QTL exceeded that obtained by the multiple QTL model in both generations. Since QTL may act together in the expression of a trait, an individual QTL could be explaining part of the variation that is due to others QTL affecting that trait. Also, interactions between QTL as those obtained in this study (linkage groups A and E) may be reflecting on the total variation explained by QTL.

As expected, alleles from the taller parent (PI) increased plant height in all QTL in both generations. In the  $F_{2.3}$  population, gene action at individual QTL varied from overdominance on linkage groups A, B, and F, complete and partial dominance

on linkage groups D and E, respectively, and additive on linkage group H. At QTL on linkage group D and H, the dominant alleles were derived from the parent CK and contributed to reduced plant height. At the remaining QTL, the dominant alleles were from PI and contributed to increased PH. Partial dominance has been reported for the *Dw* loci, which control most of the variation for PH (Quinby and Karper, 1954; Hadley, 1957). Although we identified QTL with this mode of action, most of them showed overdominance. One possible explanation for these findings may be the phenomena called pseudo-overdominance (Moll et al., 1964). With our data, verifying it is not possible if there is more than one QTL between two significantly associated RFLP loci affecting PH. If two QTL were linked in a repulsion phase, their added positive effects could result in larger estimates of dominance and lead to the apparent overdominance observed for some QTL.

#### **Correspondence between plant height QTL and *Dw* loci**

Plant height in sorghum is composed by internode length, peduncle length, node number, and panicle length (Doggett, 1988). Internode length (equivalent to PHUN in our study) is controlled by four unlinked loci, *dw1*, *dw2*, *dw3*, and *dw4* (Quinby and Karper, 1954). Linked or pleiotropic effects at some *Dw* loci have been documented for several traits such as TN, SWT, PAL, number of seeds per panicle, main panicle yield, total yield, and leaf area (Karper, 1932; Quinby and Karper, 1954; Casady, 1965; Schertz, 1973; Graham and Lessman, 1966). Robertson (1985) has proposed that alleles affecting qualitative and quantitative variation

reside at the same loci. Evidence supporting this theory has been gathered by the identification of close correspondence between genetic map location of QTL for PH and location of mutants affecting height in maize (Beavis et al., 1991; Edwards et al 1992; Doebley and Stec 1993; Veldboom et al., 1994, Veldboom and Lee, 1995) and in sorghum (Pereira and Lee, 1995; Lin et al., 1995). Additional evidence for these relationships was gathered for the *Dw* loci in this study. Unfortunately, comparisons between the map locations of *Dw* loci and PH QTL on the RFLP linkage map could not be done directly because a complete genetic linkage map has not been assembled in sorghum. Nor have all the *Dw* loci been mapped relative to RFLP loci. However, comparisons were made indirectly, by relating linked or pleiotropic effects of the *Dw* loci with PH QTL. A *dw* locus and a QTL for PH were considered associated if the *dw* locus showed pleiotropic or linked effects for a trait, and this trait had a QTL mapped at the same region as PH.

The PH QTL located on linkage group A might correspond with the *dw3* locus. Pleiotropic or linked effects on the number of seeds per panicle, SWT, PAL, PEL, TN, and LL were reported for the *dw3* locus (Casady, 1965; Schertz, 1973). Quantitative trait loci for three of these traits (PEL, PAL, and TN) were associated with PH QTL on linkage group A. Supporting the association between *dw3* locus and PH QTL on linkage group A was the fact that only this locus showed linked or pleiotropic effects for TN (Hadley, 1957). Likewise, QTL for TN had the larger effect on linkage group A and was linked around 25 cM from PH.

The PH QTL on linkage group H might correspond with the *dw2* locus. This locus showed linked or pleiotropic effects for leaf area, PAL, SWT (Graham and Lessman, 1966). Quantitative trait loci for PAL and SWT were associated with PH QTL on linkage group H. Leaf area was not considered in this present study, but QTL for leaf dimension (LL and LW) were also identified to correspond with PH on this linkage group. Additional evidence for the association between *dw2* and PH QTL on linkage group H is the linkage between the maturity (*ma1*) and *dw2* locus. These loci are linked by 8 cM (Quinby and Karper, 1945). Coincidentally, QTL for PH and anthesis were linked by approximately 3 cM only on linkage group H. Lin et al. (1995) also associated close linked QTL for PH and anthesis with the *dw2* and maturity *ma1* loci, respectively. These associations were based on the genetic effects of these QTL and the fact that they were mapped on introgressed regions supposedly harboring *dw2* and maturity *ma1* loci.

The QTL on linkage groups F may correspond with the *dw4* locus. In contrast to *dw2* and *dw3* loci, pleiotropic effects have not been reported for the morphological traits evaluated by Karper (1932), and Quinby and Karper (1954) for *dw4* locus. In this present study, PAW and LL were associated with the PH QTL on linkage group F. However, pleiotropy was not investigated for PAW and LL in these previous studies. On the other hand, the effect of *dw1* locus on traits besides PHUN is yet to be studied. By exclusion, *dw1* may be associated with the QTL on linkage group E. Plant height QTL on linkage groups B and D may not be associated with

*Dw* loci because PHUN (controlled by the *Dw* loci) was not located on these linkage groups.

Corroborating for the correspondences described between *Dw* loci and PH QTL are the similarities between the magnitude of effects of these QTL and the effects reported for the *Dw* loci. According to House (1985), complete substitution of recessive for dominant alleles at any of the four loci could decrease the height by 50 cm, but additional substitution at the remaining loci result in less reduction in PH. Unequal estimates of QTL effects, ranging from 21 to 50 cm were also observed in our study (Table 3). The correspondences defined for *Dw* loci and PH QTL may have a direct application in the conversion of exotic tropical germplasm into temperate adapted varieties. More details about the use of this information for marker-assisted backcrossed breeding will be discussed in a section addressing this subject.

#### **Orthologous genomic regions affecting height in sorghum and maize**

The possibilities of applying the more advanced genetic technology of maize to sorghum and the search for evolutionary relationships between these species have stimulated comparisons of their genomes to identify if they share similarities in gene content and function. Evidence of common gene content and function was reported by Pereira and Lee (1995), who identified three conserved regions between sorghum and maize affecting PH in both species. Similar results were also reported by Lin et al. (1995) who identified four conserved regions affecting PH in



both species. In this present study, the same number (four) of conserved regions (linkage groups A, D, E, and H) affecting height between sorghum and maize were identified, three of them at the same regions identified by Pereira and Lee (1995; Figure. 3).

*The sorghum PH QTL on linkage A may correspond with a QTL for height on the long arm of chromosome 1 of maize (Figure 3A). The genomic regions affecting this trait share four RFLP loci with one of them located in the one-LOD support interval defining these QTL. Many authors have identified QTL for PH on chromosome 1 of maize (Beavis et al., 1991; Edwards et al., 1992; Veldboom et al., 1994; Veldboom and Lee, 1995). Interestingly, this region had the largest effect on PH in maize (Veldboom et al., 1994; Veldboom and Lee, 1995), in a cross between maize and teosinte (Doebley and Stec, 1991), and in sorghum (Pereira and Lee, 1995). These findings, though exploratory, suggest that these QTL are orthologous and may have preserved their function during evolution. A QTL for anthesis with a large effect was also identified in the same region with PH on the long arm of chromosome 1 (Veldboom et al., 1994; Veldboom and Lee, 1995). It may correspond with a QTL for anthesis mapped on linkage group A in the F<sub>2</sub> generation of sorghum. In contrast to QTL in maize, this QTL had a small effect.*

Another important feature of the PH QTL on chromosome 1 of maize is the proximity of its map location relative to qualitative mutant loci *br1* and *an1* (Beavis et al., 1991; Veldboom et al., 1994; Veldboom and Lee, 1995). The *an1* mutant has a

phenotype of an andromoneoicous, gibberellin responsive dwarf with short broad leaves and few tassel branches (Emerson and Emerson, 1922; Neuffer et al., 1968). The *An1* gene has been cloned and its biochemical basis characterized (Bensen et al., 1995). Also, an RFLP marker loci derived from *an1* has been mapped linked to PH QTL on chromosome 1 of maize (Veldboom et al., 1995). In sorghum, map location of PH QTL on linkage A coincided at the same region of QTL for traits related to panicle and leaf morphology (PAL, PAW, SBL, SSB, and LW; Figure 2). Also, on linkage group A near the QTL for PH we identified a QTL for TN. A QTL for this trait was identified on the long arm of chromosome 1 of maize by Doebley and Stec (1991). These authors associated this QTL with the mutant *tb1* (teosinte branched), which produces tillers protusely and has long primary lateral branches tipped by tassel. Our population was segregating for this trait and seems to be related with progenies that have greater number of tillers. Therefore, *tb1* could be associated with the QTL for TN on linkage group A of sorghum.

Genomic regions identifying a QTL for PH on linkage group H may correspond with a region affecting plant height on the short arm of chromosome 9 of maize (Figure 3B). These regions are identified by six common RFLP loci with three in conserved and three in inverted order. On chromosome 9, QTL for PH (Beavis et al., 1991; Doebley and Stec, 1991; Edwards et al., 1992) and anthesis (Veldboom et al., 1994; Veldboom and Lee, 1995) have been located. A QTL for anthesis was also located on linkage group H and may correspond with that identified in maize.

In all instances, relatively large effects were reported for PH and small for anthesis.

*In sorghum large effects were observed for PH and anthesis on linkage group H.*

Corresponding with the map location of PH QTL on chromosome 9 are the *d3* mutant loci (Beavis et al 1991; Edwards et al., 1992). This mutant has a phenotype defined by dwarf, thick and broad leaves, and a compact tassel (Neuffer et al., 1968). Traits related to panicle and leaf morphology in sorghum such as PAL, PAW, SBL, SSBL, LL, and LW were associated at the same region with PH QTL on linkage group H. In this linkage group most of the traits were very closely linked to PH and appear to be controlled by the same QTL or set of linked genes.

The sorghum PH QTL on linkage group E may correspond with a QTL for height on chromosome 6 of maize (Figure 3C). The regions defining these QTL had only one RFLP loci in common. On chromosome 6, PH QTL (Edwards et al., 1992; Veldboom et al., 1994; Veldboom and Lee, 1995) and anthesis (Veldboom et al., 1994; Veldboom and Lee, 1995) have been reported. A QTL for anthesis was also located on linkage group E and may correspond with that identified in maize.

Corresponding with PH QTL on chromosome 6 is the *py1* mutant loci (Veldboom et al., 1994). This mutant, besides height affects also the length of the leaves (Coe et al., 1990). In sorghum, the QTL for PH on linkage group E was not associated with leaf dimensions; but with PAL and SCI.

Genomic regions affecting PH on linkage group D may correspond with a genomic region affecting height on chromosome 5 of maize (Figure 3D). These

regions had two RFLP loci in common. Plant height QTL were identified on chromosome 5 by Beavis et al. (1991). These authors, based on an integrated maize map, assigned mutant loci *na2*, *td1* and *bv1* in the same genomic region affecting height. These mutants have a phenotype defined by: *na2* erect dwarf, no response to gibberellins, and large leaves, *td1* dwarf and thick tassels, and *bv1* dwarf and short internodes (Coe et al., 1990). The PH QTL on linkage group D was associated with QTL for panicle morphology (PAL and PAW) and LL. These putative orthologous regions between sorghum and maize may have preserved a set of loci with a constellation of alleles able to display linked and/or pleiotropic effects and cause variation in the phenotype expression of these traits. The identification of the maintenance of function on conserved regions between sorghum and maize and in other members of the Gramineae family (Ahn et al., 1993; Van Deynze et al., 1995) may be very important for the transference of genetic technology among species.

#### **Use of marker-assisted backcross breeding for sorghum conversion**

To broaden the genetic base of the U.S. sorghum germplasm pool, a sorghum conversion program has been established to convert exotic tropical varieties into short stature, day-neutral genotypes adapted to conditions of mechanically harvested and temperate climates (Miller, 1982; Duncan et al., 1991). Tropical varieties of sorghum are usually too late to mature seeds and too tall to be harvested mechanically when grown in the temperate zones. Conversion is done by

introgressing one maturity gene (*ma1*) and two plant height genes via backcrossing from temperate domesticate lines into exotic materials (Poehlman, 1987).

Backcross programs can be accelerated by marker-assisted selection (MAS; Paterson et al., 1991; Hospital et al., 1992; Openshaw et al., 1994). Results obtained in this study encourage the incorporation of MAS in the ongoing sorghum conversion program. In this population, QTL for plant height explained most of the variation for this trait. The *dw2* and *ma1* loci are linked (Quinby and Karper, 1945) and may correspond to QTL for plant height and flowering date, respectively, located on linkage group H. This plant height QTL and the one on linkage group A explained the largest variation and have the largest additive effects for height. It is possible that these three QTL may correspond with the *ma1* and the dwarf genes that are being introgressed to convert the exotic germplasm. Together with phenotypic selection, MAS could lead to the ultimate aim of the conversion program, that is, to introgress as many genes as possible from the exotic material while maintaining plant stature and maturity compatible with the actual U.S. sorghum production system.

#### References

- Ahn, S., Anderson, J.A., and Tanksley, S.D., 1993. Homoeologous relationships of rice, wheat and maize chromosomes. *Mol. Gen. Genet.*, 241:483-490.
- Beavis, W.D., Grant, D., Albertsen, M., and Fincher, R., 1991. Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. *Theor. Appl. Genet.*, 83:141-145.

- Beavis, W.D., Smith, O.S., Grant, D., and Fincher, R., 1994. Identification of quantitative trait loci using a small sample of topcrossed and  $F_4$  progeny from maize. *Crop Sci.*, 34:882-896.
- Bensen, R.J., Johal, G.S., Crane, V.C., Tossberg, J.T., Schnable, P.S., Meeley, R.B., and Briggs, S.P., 1995. Cloning and characterization of the maize *An1* gene. *Plant Cell*, 7:75-84.
- Berhan, A.M., Hulbert, S.H., Butler, L.G., and Bennetzen, J.L., 1993. Structure and evolution of the genomes of *Sorghum bicolor* and *Zea mays*. *Theor. Appl. Genet.*, 86:598-604.
- Casady A.J., 1965. Effect of single height (Dw) gene of sorghum on grain yield, grain yield components, and test weight. *Crop Sci.*, 5:385-389.
- Chittenden, L.M., Schertz, K.F., Lin, Y.R., Wing, R.A., and Paterson, A.H., 1994. A detailed RFLP map of *Sorghum bicolor* X *S. Propinquum*, suitable for high-density mapping, suggests ancestral duplication of Sorghum chromosomes or chromosomal segments. *Theor. Appl. Genet.*, 87:925-933.
- Coe, E.H., Hoisington, D.A., and Neuffer, M.G., 1990. Linkage map of corn (*Zea mays* L.), pp. 6.39-6.67. In *Genetic Maps*, edited by S.J. O'Brien. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Cochran, W.G., and Cox, G.M., 1957. *Experimental designs*. John Wiley & Sons, N.Y.
- Cowen, N.M., 1988. The use of replicated progenies in marker-based mapping of QTLs. *Theor. Appl. Genet.*, 75:857-862.
- deWet, J.M.C., 1978. Systematics and evolution of *Sorghum* sect. *Sorghum* (*gramineae*). *Amer. J. Bot.*, 65:477-484.
- Doebley, J., and Stec, A., 1991. Genetic analysis of the morphological differences between maize and teosinte. *Genetics*, 129:285-295.
- Doebley, J., and Stec, A., 1993. Inheritance of morphological differences between maize and teosinte: comparison of results for two  $F_2$  populations. *Genetics*, 134:559-570.
- Doggett, H., 1988. *Sorghum*. Longman Group U.K. Ltd., Essex, England

- Duncan, R., Bramel-Cox, P.J., and Miller, F.R., 1991. Contributions of introduced sorghum germplasm to hybrid development in the USA. (CSSA special publication no. 17:69-101).
- Edwards, M.D., Helentjaris, T., Wright, S., and Stuber, C.W., 1992. Molecular-marker-facilitated investigations of quantitative trait loci in maize. *Theor. Appl. Genet.*, 83:765-774.
- Edwards, M.D., Stuber, C.W., and Wendel, J.F., 1987. Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. *Genetics*, 116:113-125.
- Emerson, R.A., and Emerson, S.H., 1992. Interactions of two andromonoecious types of maize, dwarf and anther ear. *Genetics*, 7:203-236.
- Fatokun, C.A., Menancio-Hautea, D.I., Danesh, D., and Young, N.D., 1992. Evidence for orthologous seed weight genes in cowpea and mung bean based on RFLP mapping. *Genetics*, 132:841- 846.
- Graham, D., and Lessman, K.J., 1966. Effects of height on yield and yield components of two isogenic lines of *Sorghum vulgare* Pers. *Crop Sci.*, 6:372-374.
- Hadley, H.H., 1957. An analysis of variation in height in sorghum. *Agron. Jour.*, 49:144-147.
- Hallauer, A.R., and Miranda, J.B., 1988. *Quantitative genetics in maize breeding* 2nd ed., Iowa State University Press, Ames, Iowa.
- Hammer, G.L. and Vanderlip, R.L., 1989. Genotype-by-environment interaction in grain sorghum. III. Modeling the impact in field environments. *Crop Sci.*, 29:385-391.
- House, L.R., 1985. A guide to sorghum breeding. Second edition. ICRISAT, Patancheru, India.
- Hospital, F., Chevalet, C., and Mulsant, P., 1992. Using markers in gene introgression breeding programs. *Genetics*, 132:1199-1210.

- Hulbert, S.H. Richter, T.E. Axtell, J.D., and Benetzen, J.L., 1990. Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. *Proc. Natl. Acad. Sci. U.S.A.*, 87:4251-4255.
- Lander, E.S., and Botstein, D., 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, 121:185-199.
- Lin, Y.R., Schertz, K.F., and Paterson, A. H., 1995. Comparative analysis of QTLs affecting plant height and maturity across the *Poaceae*, in reference to an interspecific sorghum population. *Genetics*, 141:391-411.
- Jansen, R.C., and Stam, P., 1994. High resolution of quantitative traits into multiple loci via interval mapping. *Genetics*, 136:1447-1455.
- Karper, R.E., 1932. A dominant mutation of frequent recurrence in sorghum. *Amer. Nat.*, 66:511-529.
- Knapp, S.J., Stroup, W.W., and Ross, W.M., 1985. Exact confidence intervals for heritability on a progeny mean basis. *Crop Sci.*, 25:192-194.
- Knott, S.A., and Haley, C.S., 1992. Aspects of maximum likelihood methods for the mapping of quantitative trait loci in line crosses. *Genet. Res.*, 60:139-151.
- Neuffer, M.G., Jones, L., and Zuber, M.S., 1968. The mutants of maize. *Crop Sci. Soc. of Amer.* Madison, Wisconsin.
- Mather, K., and Jinks, J.L., 1971. *Biometrical genetics*. Chapman and Hall, London.
- Miller, F.R., 1982. Genetics and environmental response characteristics of sorghum. In: House, L.R., Munghogho, L.K. and Peacock, J.M. (eds). *Sorghum in the Eighties*. ICRISAT, Patancheru, India, pp. 393-402.
- Moll R.H., Lindsey, M.F, and Robinson, H.F., 1964. Estimates of genetic variances and level of dominance in maize. *Genetics*, 49:411-423.
- Openshaw, S.J., Jarboe, S.G., and Beavis, W.D., 1994. Marker-assisted selection in backcross breeding. In: *Proceedings of the symposium analysis of molecular marker data*. Joint plant breeding symposia series. American Society for Horticultural Science/Crop Science Society of America. pp. 41-43.



- Paterson, A.H., Damon, S., Hewitt, J.D., Zamir, D., Rabinowitch, H.D., Lincoln, S.E., Lander, E.S., and Tanksley, S.D., 1991. Mendelian factors underlying quantitative traits in tomato: Comparison across species, generations and environments. *Genetics*, 127:181-197.
- Pereira, M.G., Lee, M., Bramel-Cox, P., Woodman, W., Doebley, J., and Whitkus, R., 1994. Construction of an RFLP map in sorghum and comparative mapping in maize. *Genome*, 37:236-243.
- Pereira, M.G., and Lee, M., 1995. Identification of genomic regions affecting plant height in sorghum and maize. *Theor. Appl. Genet.*, 90:380-388.
- Pereira, M.G., Ahnert, D., Lee, M., and Klier, K., 1995. Genetic mapping of quantitative trait loci for panicle characteristics and seed weight in sorghum. *Rev. Brasil. Genet.*, 18:249-257.
- Poehlman, J.M., 1987. *Breeding sorghum and millet*. In: breeding field crops. AVI Pub. Co., Westport, Connecticut. pp. 308-358.
- Quinby, J.R., and Karper, R.E., 1945. The inheritance of three genes that influence time of floral initiation and maturity date in Milo. *J. Amer. Soc. Agron.* 37:916-936.
- Quinby, J.R. and Karper, R.E., 1954. Inheritance of height in sorghum. *Agron. J.*, 46:211-216.
- Robertson, D.S., 1985. A possible technique for isolating genic DNA for quantitative traits in plants. *J. Theor. Biol.*, 117:1-10.
- SAS Institute., 1988. SAS introductory guide for personal computers. SAS Institute, Inc., Cary, North Caroline.
- Shapiro, S.S. and Wilk, M.B., 1965. An analysis of variance for normality (complete samples). *Biometrika*, 52:591-611.
- Stuber, C.W., Edwards, M.D., Wendel, J.F., 1987. Molecular marker-facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component traits. *Crop Sci.*, 27:639-648.

- Stuber, C.W., Lincoln, S.E., Wolff, D.W., Helentjaris, T., and Lander E.S., 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics*, 132:823-839.
- Schön, C.C., Lee, M., Melchinger, A.E., Guthrie, W.D., and Woodman, W.L., 1993. Mapping and characterization of quantitative trait loci affecting resistance against second-generation European corn borer in maize with the aid of RFLPs. *Heredity*, 70:648-659.
- Schertz, K.F., 1973. Single height-gene effects in hybrids of double-haploid *Sorghum bicolor* (L.) Moench. *Crop Sci.*, 13:421-423.
- Soller, M. and Beckmann, J.S., 1990. Marker-based mapping of quantitative trait loci using replicated progenies. *Theor. Appl. Genet.*, 80:205-208.
- Searle, S.R. (1971). *Linear models*. John Wiley and Sons, New York.
- Van Deynze, A.E., Dubcovsky, J., Gill, K.S., Nelson, J.C., Sorrells, M.E., Dvorak, J., Gill, B.S., Lagudah, E.S., McCouch, S.R., and Appels, R., 1995. Molecular-genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. *Genome*, 38:45-59.
- Veldboom, L.R., Lee, M., and Woodman, W. L., 1994. Molecular marker-facilitated studies in an elite maize population: I. Linkage analysis and determination of QTL for morphological traits. *Theor. Appl. Genet.*, 88:7-16.
- Veldboom, L.R. and Lee, M., 1995. Genetic mapping of morphological traits in maize in stress and nonstress environments. *Crop Sci.* (In review).
- Whitkus, R., Doebley, J., and Lee, M., 1992. Comparative genome mapping of sorghum and maize. *Genetics*, 132:1119-1130.

Table 1. Means, ranges, genetic variance components, and heritabilities for 152  $F_{2,3}$  sorghum lines.

	Plant height (cm)
<b>Means and ranges</b>	
$F_{2,3}$ progenies	286.8 (114.6 - 353.8)
PI229828	312.0 (306.1 - 318.0)
CK60	93.9 (89.8 - 98.1)
$F_1$	298.4 (298.4 - 312.8)
<b>Variance components</b>	
$F_{2,3}$ progenies	
$\sigma_g^2 \pm \text{SE}$	1114.8 $\pm$ 142.8**
$\sigma^2$	254.2
Parents	
$\sigma_p^2 \pm \text{SE}$	24588.2 $\pm$ 20179.9**
$F_1$ 's	
$\sigma_e^2$	207.9 $\pm$ 86.3 <sup>ns</sup>
<b>Heritability (<math>h^2</math>) and 90% C.I. of <math>h^2</math></b>	
$F_{2,3}$ progenies	0.90 (0.86 - 0.92)

\*\* Significant at 0.01 probability level.

<sup>ns</sup> Nonsignificant at 0.01 probability level.

Table 2. Genetic location and effects of QTL affecting plant height for 152  $F_{2:3}$  sorghum lines.

Linkage group	interval <sup>a</sup>	R <sup>2</sup> (%) <sup>b</sup>	LOD <sup>c</sup> Score	Genetic Additive	Effects <sup>d</sup> Dominance	d/a	Gene action	Direc-tions <sup>e</sup>
A	ISU123 ISU116	30	8.5	26.4	40.1	1.5	OD	PI
B	ISU155 UMC71	6	2.2	11.3	20.1	1.8	OD	PI
D	ISU126A ISU042	24	2.7	8.4	-9.8	-1.1	D	PI
E	ISU140 PIO100016	24	7.0	25.1	16.4	0.7	PD	PI
F	ISU071 ISU130	12	2.5	5.8	12.3	2.1	OD	PI
H	ISU032B ISU056	19	5.9	19.6	-2.2	-0.1	A	PI
Total		77.5	36.4					

<sup>a</sup> Flanking markers of the most likely QTL position.

<sup>b</sup> Percentage of phenotypic variation explained by QTL.

<sup>c</sup> LOD threshold = 2.4.

<sup>d</sup> Genetic effects are associated with the allele from PI229828. Thus, a negative value means that the PI229828 allele is decreasing the value of the trait.

<sup>e</sup> Direction of response is the parent whose additive value of a loci increases the value of the trait.

Table 3. Genotypic class averages of QTL affecting plant height for 152  $F_{2,3}$  sorghum lines.

Linkage	Nearest <sup>a</sup>	Genotypic Classes <sup>b</sup>		
group	locus	CK/CK( $\mu$ AA)	CK/PI( $\mu$ AB)	PI/PI( $\mu$ BB)
		-----cm-----		
A	ISU116	248	290	299
B	ISU155	263	290	289
D	ISU042	272	290	293
E	ISU140	259	292	298
F	ISU130	269	289	293
H	ISU032B	275	286	319

<sup>a</sup> Nearest marker of the most likely QTL position.

<sup>b</sup> Trait means for homozygous ( $\mu$ AA) CK60, heterozygous ( $\mu$ AB) CK60/PI229828, and homozygous ( $\mu$ BB)PI229828 alleles at QTL affecting height.

Table 4. Digenic interaction between QTL from linkage groups A (isu116) and E (isu140) in 152  $F_{2:3}$  sorghum lines.

Genotype at isu140	Genetic effects isu116		Genotype at isu116	Genetic effects isu140	
	a <sup>a</sup>	d <sup>b</sup>		a <sup>a</sup>	d <sup>b</sup>
CK60/CK60	51	52	CK60/CK60	54	21
CK60/PI <sup>c</sup>	31	14	CK60/PI	17	1
PI/PI	12	19	PI/PI	15	20

<sup>a</sup> additive effects in cm.

<sup>b</sup> dominance effects in cm.

<sup>c</sup> PI229828.

Table 5. Genomic locations and percentage of phenotypic variation of QTL for fourteen traits mapped on linkage groups A, B, D, E, F, and H with plant height in 152  $F_2$  plants and their  $F_{2.3}$  progenies.

Linkage Group	Interval <sup>a</sup>		R <sup>2</sup> (%) <sup>b</sup>		Direction <sup>c</sup>
	F <sub>2-3</sub>	F <sub>2</sub>	F <sub>2-3</sub>	F <sub>2</sub>	
Height to the uppermost node (cm)					
A	isu123-isu116		41		PI
E	isu140-pio10016		19		PI
F	ISU071-isu130		11		PI
H	isu032B-isu156		19		PI
Peduncle length (cm)					
A	isu123-isu116		30		CK
B	umc71-isu058		18		PI
Number of nodes (number)					
A	isu116-isu074		17		PI
D	pio20608-isu107		9		PI
F	isu037-isu117		8		CK
H	isu032B-isu116		33		PI
Panicle length (cm)					
A	isu123-isu116	isu116-isu074	28	25	CK
B	isu071-isu058	isu058-isu030	9	9	PI
D	bnl5.40-pio20566		8		PI
E	isu140-pio1016		9		PI
H	isu032B-isu116	isu032B-isu116	15	15	PI
Panicle width (cm)					
A	isu123-isu116	isu116-isu074	21	30	CK
D	isu042-npi104A		11		PI
F	isu130-npi400		8		CK
H	pio10005-isu032B	isu032B-isu116	14	16	PI

<sup>a</sup> Flanking markers of the most likely QTL position.

<sup>b</sup> Percentage of phenotypic variance explained by individual QTL.

<sup>c</sup> Direction of response is the parent whose additive value of a loci increases the value of the trait (PI = PI229828; CK = CK60).

Table 5. (continued)

Linkage Group	Interval		R <sup>2</sup> (%)		Direction
	F <sub>2,3</sub>	F <sub>2</sub>	F <sub>2,3</sub>	F <sub>2</sub>	
Leaf length (cm)					
B	isu058-isu030	isu058-isu030	17	13	PI
D	isu126A-isu042	isu126A-isu042	11	12	PI
F	isu151-isu071		7		CK
H	isu156-isu030	isu032B-isu156	19	21	PI
Leaf width (cm)					
A	isu095-umc33		15		CK
B	isu138-isu126B		15		CK
H		pio10005-isu032B		10	PI
Stalk circumference (cm)					
A	umc33-isu123	isu123-isu116	48	27	CK
B	isu147-isu155		16		CK
E	pio10016-pio20714		9		CK
H		isu136-pio10005		10	PI
Number of tillers (number)					
A	umc33-isu123	isu123-isu116	27	8	PI
B		pio200568-isu147		8	PI
B		isu138-isu126B		19	PI
D	isu080-isu056		11		CK
Anthesis (GDD)					
A		isu074-isu152		11	PI
E	isu026-isu064		8		CK
F	isu037-isu117		7		CK
H	isu032B-isu156	isu032B-isu156	41	18	PI
Peduncle diameter (mm)					
A		isu123-isu1116		9	CK
B		umc71-isu058		9	PI
F		isu115-bnl6.20		9	PI
H		umc114-isu110		8	PI



Table 5. (Continued)

Linkage Group	Interval		R <sup>2</sup> (%)		Direction
	F <sub>2:3</sub>	F <sub>2</sub>	F <sub>2:3</sub>	F <sub>2</sub>	
Seed-branches/panicle (number)					
A		isu116-isu074	9		PI
F		isu117-isu086	14		PI
See-branch length (cm)					
A		isu116-isu074	37		CK
H		isu032B-isu156	17		PI
Length of the sterile portion of the seed-branch (cm)					
A		isu116-isu074	17		CK
H		isu032B-isu156	17		PI
Seed weight (g)					
E		isu126-umc74	9		PI
H		isu156-isu034	16		CK

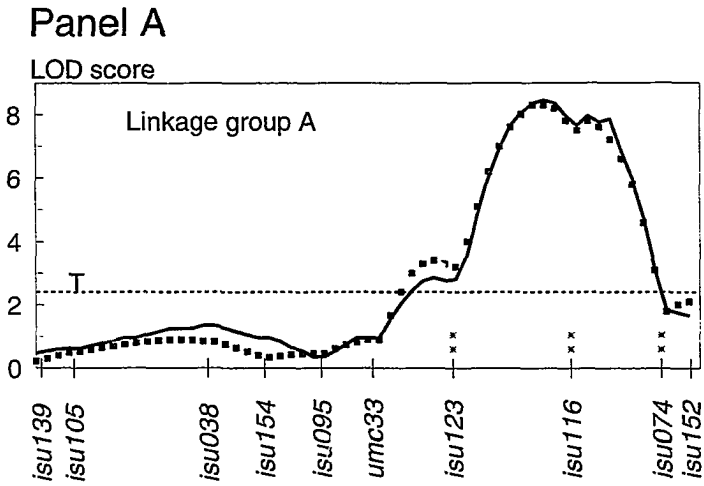


Figure 1. Panels A-F show the log-likelihood plots of linkage groups A, B, D, E, F, and H for plant height. The plots of LOD values for  $F_2$  plants and their  $F_3$  progeny are indicated by dotted (---) and solid lines (—) lines, respectively. Vertical lines represent LOD score, horizontal dotted lines (T) indicate the position for the LOD threshold (2.4), and \*\* represent loci significantly associated with QTL at 0.01 level of probability for SFAOV.

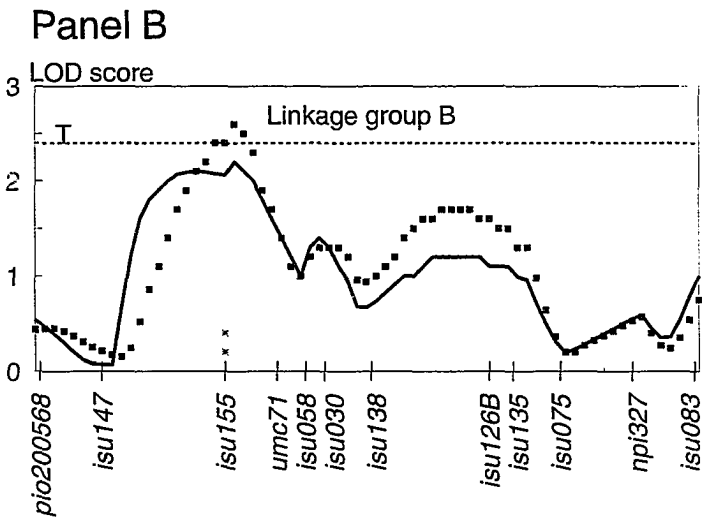


Figure 1. (continued)

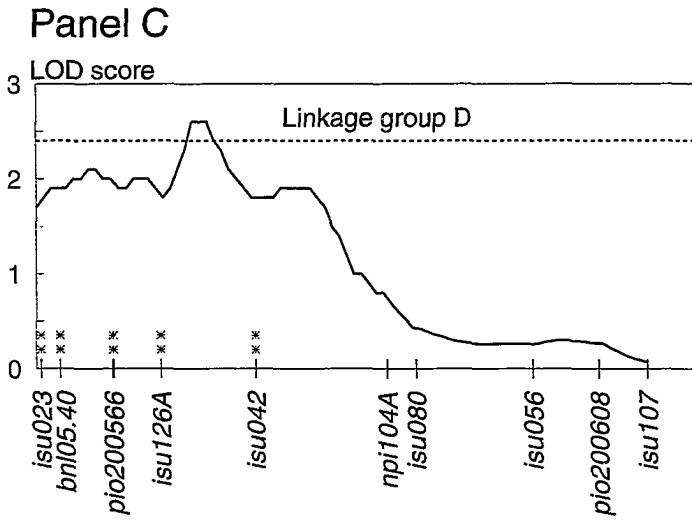


Figure 1. (continued)

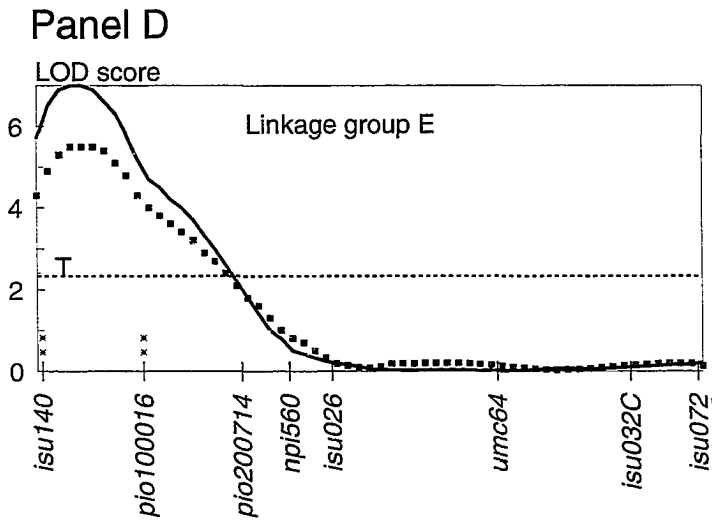


Figure 1. (continued)

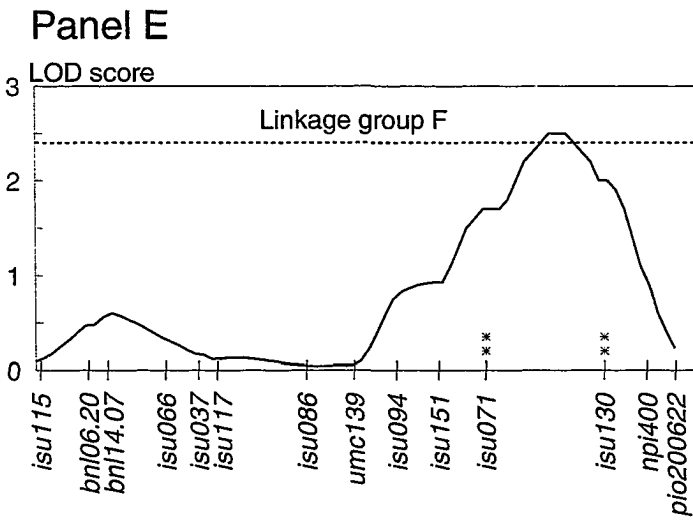


Figure 1. (continued)

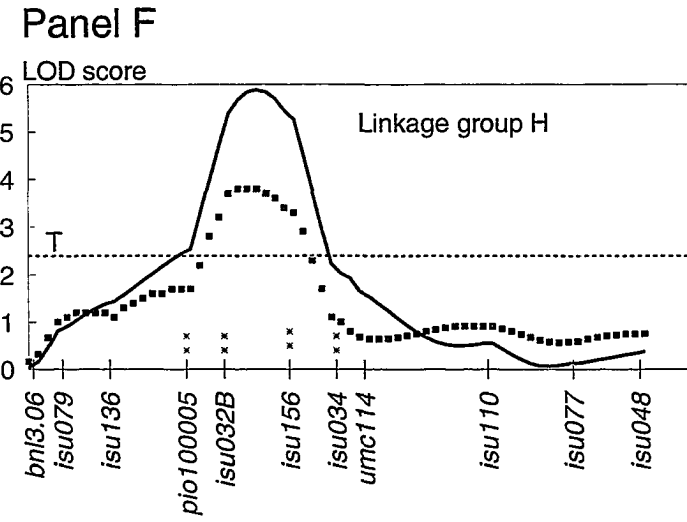


Figure 1. (continued)

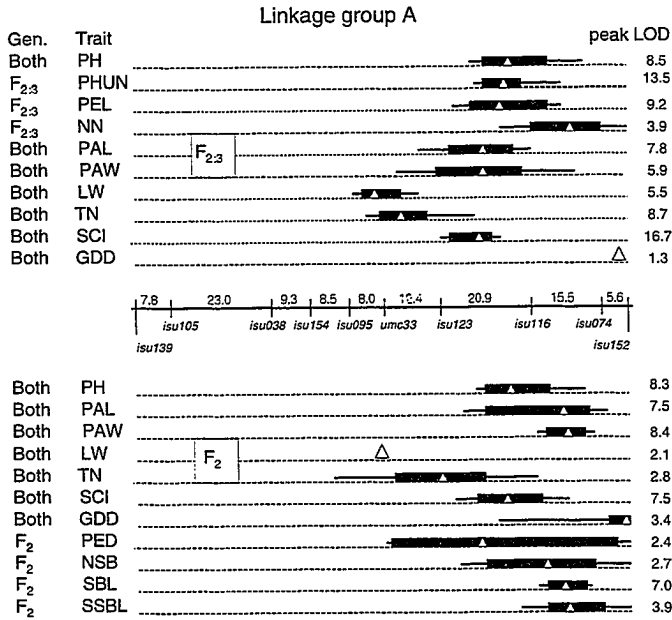


Figure 2. Linkage group location of QTL for plant height and QTL for traits associated with plant height at the same linkage group in the CK60 X PI229828 F<sub>2</sub> plants and their F<sub>2.3</sub> progenies. QTL positions are indicated by the shaded triangle with their one-LOD (thick black lines) and two-LOD (thin black lines) support intervals. When a QTL was located in one generation but not in the other, the highest LOD peak (although with a LOD < 2.4) is indicated by an open triangle for the other population. Trait acronyms are indicated in the Materials and Methods section. Traits as PHUN, NN, and PEL were only recorded in the F<sub>2.3</sub> progenies whereas PDI, NSB, SBL, SSBL and SWT were recorded only in the F<sub>2</sub> plants. The remaining traits were recorded in both generations.



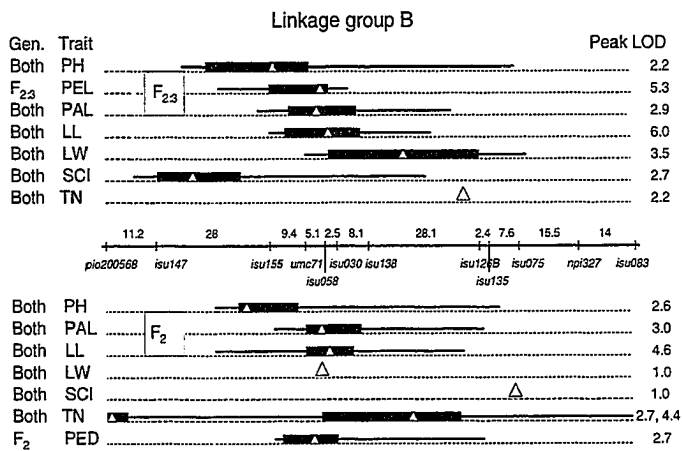


Figure 2. (continued)

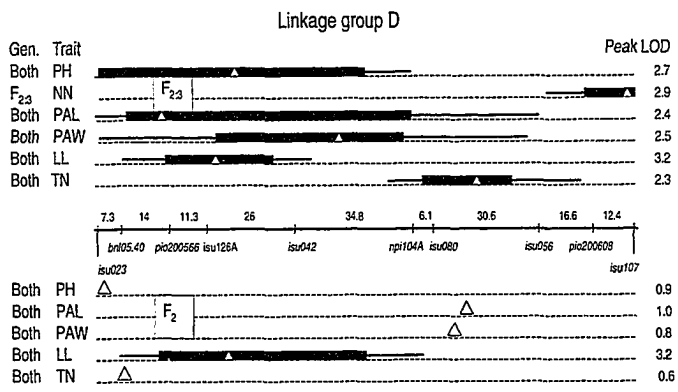


Figure 2. (continued)

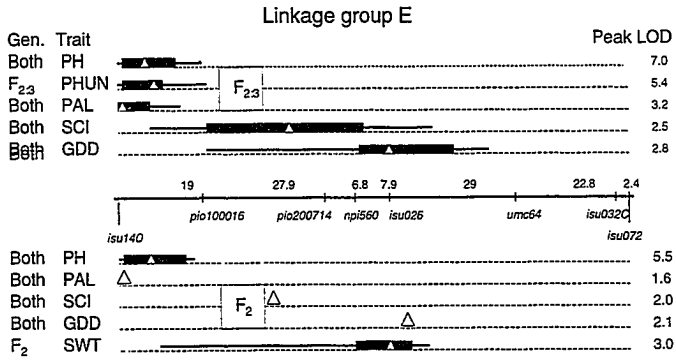


Figure 2. (continued)

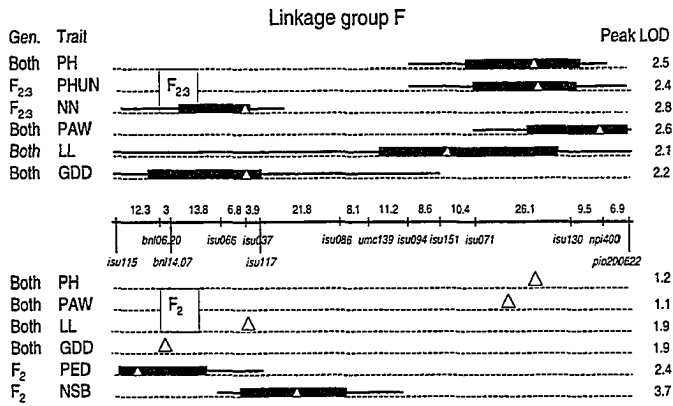


Figure 2. (continued)

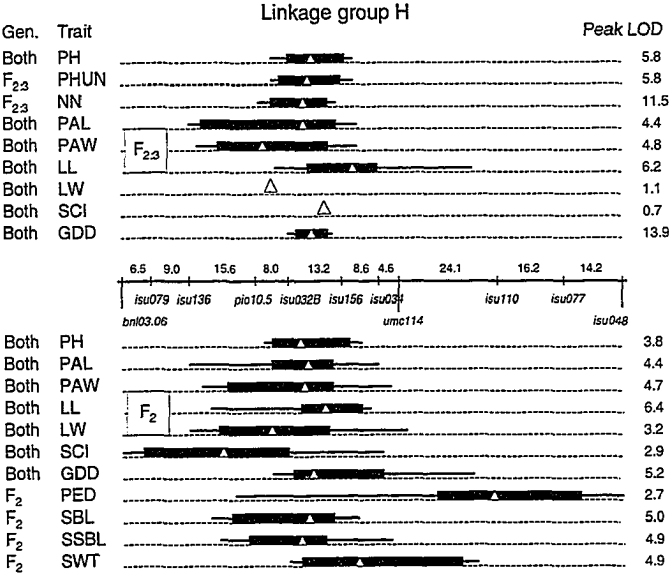


Figure 2. (continued)

## Panel A

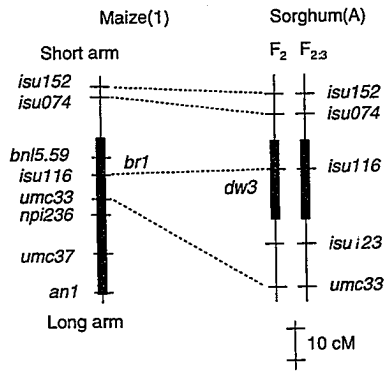


Figure 3. Comparison of genomic regions of sorghum and maize having common RFLP loci (connected by dotted lines) linked to QTL for plant height. Numbers and letters in parenthesis represent the maize chromosome number and sorghum linkage group, respectively. The shaded areas are the confidence intervals (1.0 log unit as indicated by mapmaker-QTL program) for plant height QTL. In panel A, B, and D the maize QTL were located by Beavis et al. (1991) and in panel C by Veldboom et al. (1994). *br1*, *an1*, *d3*, *py1*, *na2*, *bv1*, and *td1* represent maize loci with qualitative mutants for plant height approximate located at that genomic region (Maize Newsletter, 1993).

## Panel B

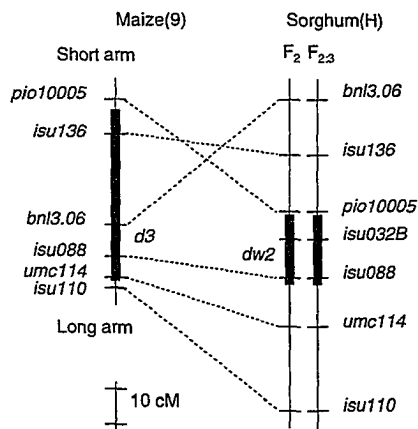


Figure 3. (Continued)

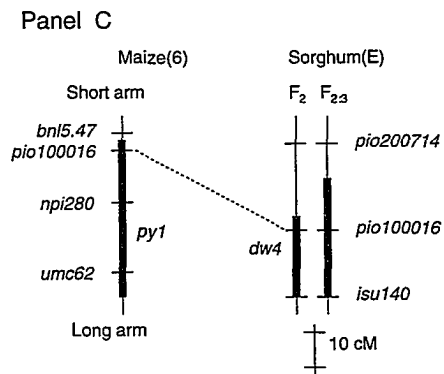


Figure 3. (Continued)



## Panel D

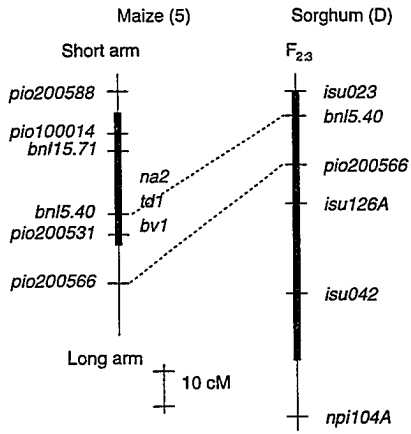


Figure 3. (Continued)

## GENETIC MAPPING OF MORPHOLOGICAL TRAITS IN SORGHUM ACROSS GENERATIONS

A paper for submission to *Crop Science*

Dario Ahnert, Michael Lee\*, and Messias G. Pereira

### Abstract

Restriction fragment length polymorphisms (RFLPs) were employed to investigate quantitative trait loci for morphological traits among 152  $F_{2,3}$  sorghum [*Sorghum bicolor* (L.) Moench] lines. These lines were obtained from the cross CK60 (*Sorghum bicolor* ssp. *bicolor*) and PI229628 (*Sorghum bicolor* ssp. *drummondii*) and grown in a replicated trial. Traits evaluated in this study included plant height, peduncle length, panicle and leaf dimensions, number of nodes and tillers, stalk circumference, and flowering date. Our objectives were to estimate genetic map location and effects of quantitative trait loci (QTL) affecting these traits in the  $F_{2,3}$  population and compare the results with those obtained in the  $F_2$  plants. In the  $F_{2,3}$ , a total of 42 QTL was identified with a range of two to seven per trait. In the  $F_2$ , 25 QTL were located with a range of two to six per trait. Usually, one QTL per trait showed large genetic effects and the remaining were of smaller effects. Gene action varied from additive or partial to overdominance. Eighty-one percent of the QTL identified in this study showed partial dominance to overdominance effects

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suggesting that nonadditive effects are very important in this population. Many genetic regions were associated with more than one trait indicating linked and/or pleiotropic effects. Most ( 64%) of the QTL detected with  $F_2$  plants coincided with regions identified with  $F_{2-3}$  progenies. Overall, QTL with the largest effects were consistent across generations and environments.

### **Introduction**

*Restriction fragment length polymorphisms (RFLPs) have been widely used as a tool for genetic studies in different crop species. Utilization of RFLPs in mapping and characterization of quantitative trait loci (QTL; Paterson et al., 1991; Beavis et al., 1991, 1994; Schön et al., 1994; Veldboom et al., 1994, Pereira and Lee, 1995; Lin et al., 1995) have been reported. In this study, RFLPs were used to locate and characterize the effects of genetic factors affecting quantitative variation for plant height, peduncle length, panicle length and width, node number, leaf length and width, tiller number, stalk circumference, and flowering date. QTL map location and effects of some of these traits were studied previously in  $F_2$  plants (Pereira and Lee, 1995; Pereira et al., 1995). Here, we report on the  $F_{2-3}$  lines obtained from the  $F_2$  plants of these previous studies.*

In sorghum, Identification and characterization of QTL have been facilitated by the development of linkage maps in this crop (Hulbert et al., 1990; Binelli et al., 1992; Whitkus et al., 1992; Berhan et al., 1993; Pereira et al., 1994, Chittenden et al., 1994). In an  $F_2$  population of 152 plants, four QTL were identified for plant

height accounting for 63% of the variation in this trait (Pereira and Lee, 1995). Two to six QTL were identified each for panicle morphology, leaf dimensions, number of tillers and nodes, stalk circumference, and flowering date (Pereira, 1993; Pereira et al., 1995). These QTL explained 26 to 69% of trait variation in multiple QTL models. In a larger and more divergent  $F_2$  population, six QTL were detected for plant height and three for flowering date explaining 71% and 87%, respectively, of the phenotypic variation (Lin et al., 1995). In  $F_{2:3}$  progenies, the same number (six) of QTL were mapped for plant height, explaining 77% of the variation in multiple models (Ahnert et al., 1995). In  $F_7$  recombinant inbred lines, three major regions were identified for drought resistance (Xu et al., 1994).

These morphological and/or agronomical traits are important to breeding and introgression programs (Poehlman, 1987; Miller, 1982; Duncan et al., 1991) and germplasm classification schemes (Snowden, 1936; Harlan and deVet, 1972) in sorghum. However, there is very limited information regarding the genetic location of genes affecting these traits. Height is affected by alleles at four unlinked loci *dw1*, *dw2*, *dw3*, and *dw4*, and some unknown factors (Quinby and Karper, 1954). Alleles at some of these loci may also affect tillering, panicle characteristics, leaf area, and yield (Casady, 1965; Graham and Lessman, 1966). Number of nodes is influenced by the maturity loci (*Ma*; Doggett, 1988). Four unlinked *Ma* loci (*ma1*, *ma2*, *ma3*, and *ma4*) have been identified to control flowering date (Quinby, 1966). Both, epistatic and dominance effects were observed at these four loci (Quinby,

1975). Also, allelic series at the *ma1* and *ma3* loci may exist (Quinby, 1972). The maturity (*ma1*) and dwarfing gene *dw2* are linked by 8 cM (Quinby and Karper, 1945). Monogenic dominant inheritance has been reported for loose to compact panicle (Doggett, 1988). Dominance (Liang and Walter, 1968) and heterosis (Kirby and Atkins, 1968; Kambal and Webster, 1966) have also been reported for morphological and maturity traits in biometrical studies in sorghum. High correlations have been reported for plant height and its components, height to the uppermost node, peduncle length, and panicle length (Kambal and Webster, 1966) and between maturity and stalk diameter (Kirby and Atkins, 1968). Mapping and characterization of the underlying factors affecting these traits would be beneficial for breeding and botanical classification purposes. Therefore, the goal of this experiment was to locate and characterize QTL for these traits.

Previously, Pereira (1993), Pereira et al. (1995), and Pereira and Lee (1995) reported QTL for plant height, flowering date, tiller number, leaf and panicle dimensions, and stalk circumference using  $F_2$  plants as the mapping population. In the present study, replicated  $F_{2,3}$  progenies were used. Replicated progenies are advantageous to  $F_2$  populations due to reduced experimental error and increasing precision of QTL mapping. Also, they allow testing in different environments and detection of QTL with smaller effects (Cowen, 1988; Soller and Backmann, 1990). The specific objectives of our study were to: I) identify location and effects of QTL for morphological and maturity traits in  $F_{2,3}$  sorghum lines, and II) compare estimates

of QTL location and effects obtained in the  $F_{2,3}$  generation to those with their parental  $F_2$  plants.

## **Material and Methods**

### **Mapping population**

One hundred and fifty-two  $F_{2,3}$  sorghum lines obtained from a cross between genotypes CK60 and PI229828 were evaluated for several morphological traits. Procedures for seed production, genetic constitution, and origin of these lines were previously described in Ahnert et al. (1995). These lines were chosen as parents because they differ in several characteristics. CK60 has susceptibility to greenbug biotype E, short stature, wide leaves, single stalk, and compact panicle. In contrast, PI229828 has resistance to greenbug biotype E, tall stature, narrow leaves, multiple tillers, and an open loose panicle.

### **Field design**

A total of 156 entries was evaluated in a 12 x 13 simple rectangular lattice design of one-row plots with two replications at the Agronomy and Agricultural Engineering Research Center near Ames, Iowa, in 1994. Details about the experimental organization and planting procedures were described in Ahnert et al. (1995). Favorable weather throughout the 1994 growing season encouraged the establishment of plots with complete stands without root or stalk lodging. Morphological traits were recorded on ten plants per plot and averaged to obtain an estimate of a trait value on a plot mean basis. Data were recorded for the following

traits:

Plant height (PH, the height of the plant measured at anthesis from the soil surface to the tip of the panicle);

Plant height to the uppermost node (PHUN, the height of the plant measured at anthesis from the soil surface to the uppermost node);

Panicle length (PAL, the length of the panicle measured at harvesting);

Number of nodes (NN; the number of nodes on the main stem counted at anthesis);

Peduncle length [PEL, the length of the peduncle obtained by the following difference:  $PH - (PAL + PHUN)$ ];

Panicle width (PAW, the width at the widest part of the panicle at harvesting);

Leaf length (LL, the length of the third leaf from the panicle at anthesis);

Leaf width (LW, the width of the widest part of the third leaf from the panicle at anthesis);

Number of tillers (TN, the number of tillers with seeds per plant counted at anthesis);

Stalk circumference (SCI, the circumference of the main stalk measured around 15 cm above the soil surface at anthesis);

Flowering date (GDD to anthesis) is the accumulated growing degree days (GDD) in °C from planting date to the date 50% of the plants in a plot reached anthesis. GDD were calculated from the daily minimum and maximum temperature

as described by Hammer and Vanderlip (1989). The minimum and maximum temperatures were 7 °C and 30 °C, respectively, if the actual temperatures exceeded these limits. All metric traits were measured to the nearest cm unless indicated otherwise.

#### **RFLP assays and map construction**

RFLP assays and the procedures for map construction were previously described in Pereira and Lee (1995). RFLP patterns of locus *isu* 147, mapped on linkage group (LG) B, were rescored and the data used to recalculate distances between loci on this LG. The rescoring did not cause major changes in distances on LG B. The RFLP linkage map is composed of ten LGs defined by 111 loci, and has a total map distance of 1307 cM with an average distance between markers of 12.9 cM.

#### **Data analysis**

Trait entry means were adjusted for lattice block effects and the effective error mean square calculated according to Cochran and Cox (1957). The adjusted trait means of the 152 progenies were tested for normality by the W-test (Shapiro and Wilk, 1965). Standard analysis of variances for a single rectangular lattice design model was performed. Sum of squares due to entry means were partitioned into sums of squares due to  $F_{2,3}$  progenies, checks and progeny versus checks.

Genotypic component of variances ( $\delta^2g$ ) estimates of the lines and the standard error (SE) were calculated as described by (Searle, 1971). Heritability was



calculated on a progeny mean basis (Hallauer and Miranda, 1988). Exact 90% confidence interval on heritability estimates were calculated according to Knapp et al. (1985). Also, the estimate of heritability using the parent-offspring regressions of the  $F_{2.3}$  on the  $F_2$  phenotypic traits were calculated as described by Hallauer and Miranda (1988). Phenotypic correlations among traits were calculated on adjusted entry means, excluding the checks.

#### **QTL identification**

Interval mapping (Lander and Botstein, 1989) and single-factor analysis of variances (SFAOV; Edwards et al., 1987) were used to estimate map position and genetic effects of QTL for all traits. Interval mapping was accomplished with MAPMAKER/QTL 1.1 (Paterson et al., 1991; Lander and Botstein, 1989) and SFAOV with a program written using SAS (SAS Institute Inc. 1988). For interval mapping, a LOD threshold of 2.4 was used to declare a presence of a QTL in a given region of the map. Considering the "sparse map" case, this threshold value corresponds to test for QTL at a probability level of 0.05 (given 101 marker intervals) genome-wise (Lander and Botstein, 1989). A single-QTL model was used to estimate individual QTL map position. In this model, one QTL at a time was allowed to explain the phenotypic variation in a trait. Once individual QTL were defined for each trait, the significant ones were added into a multiple-QTL model (Lander and Botstein, 1989), and the total phenotypic variation and genetic effects were calculated (Jansen and Stam, 1994). In cases where MAPMAKER/QTL showed

consecutive peaks (maximum likelihood QTL position) apparently identifying two QTL within a LG, the one with the largest LOD was fixed (Lander and Botstein, 1989) and a two-QTL model calculated. If the LOD for the combined model increased by 2.0 or more the LOD obtained from a one-QTL model, two QTL were declared (Stuber et al., 1992).

Estimates of dominance effects were doubled as described in Mather and Jinks (1971). Dominance effects in the  $F_{2:3}$  lines are expected to be reduced by half from heterozygous  $F_2$  plants. By doubling the dominance effects calculated in an  $F_{2:3}$  generation, these effects become expressed in an  $F_2$  basis. Average levels of dominance were calculated by the ratio  $d/a$ , where  $d$  refers to dominance effect in an  $F_2$  population, and  $a$  represents the additive effect. Gene action was based on the average level of dominance according to the criteria proposed by Stuber et al. (1987): additive (A) = 0-0.20; partial dominance (PD) = 0.21-0.80; dominance (D) = 0.81-1.20; and overdominance (OD) > 1.20.

SFAOV was performed for each pair-wise combination of quantitative trait and RFLP locus (Edwards et al., 1987). Trait data consisted of adjusted entry means. F-tests were conducted to verify if significant variation in trait expression was associated with differences among the genotypic classes at each locus. To declare linkage between a marker and a QTL, a significance level of 0.01 was used. With this level of significance, there is 1% chance of declaring a false positive (Type I error) at any single trait. The probability of declaring at least one false positive

genome-wide is  $1-(0.99)^n$ , with  $n$  equal to the number of loci.

## Results and Discussion

### Field data analysis

All traits displayed normal distributions according to the  $W$  statistics (Shapiro and Wilk, 1965), except PHUN ( $W = 0.92$ ) and SCI ( $W = 0.95$ ). PHUN's distribution was skewed toward taller values. This tendency was expected because increased height to the uppermost node is partially dominant (Quinby and Karper, 1954; Hadley, 1957; Pereira and Lee, 1995). SCI's distribution was slightly skewed towards thinner stalks. Inheritance of this trait has yet to be studied. Although interval mapping assumes normal distribution of the trait data (Lander and Botstein, 1989), minor violation of this assumption should not alter QTL identification (Stuber et al., 1992; Veldboom et al., 1994; Beavis et al., 1994).

Means, genetic variance components, and heritabilities are summarized in Table 1. PI229828 had larger values for PHUN, PAL, PAW, PEL, LL, and TN. CK60 had larger values for LW and SCI. Parental means were of similar magnitudes for NN and GDD to anthesis. Genotypic variances ( $\sigma^2_g$ ) of the  $F_{2,3}$  lines were highly significant ( $P \leq 0.01$ ) for all traits, indicating genetic differences among lines. Broad sense heritabilities in the  $F_{2,3}$  progenies were high, above 80% for all traits except tillering (60%). However, these values may be biased upwards because they were estimated from data collected in just one environment. Trait heritabilities in the  $F_2$  population, obtained from the parent-offspring regression of

the  $F_{2.3}$  on the  $F_2$ , ranged from 8% (TN) to 57% (PAL; Table 1). TN was the character with the lowest heritability estimates in  $F_2$  and  $F_{2.3}$  generations. All traits manifested transgressive segregation, and the mean of  $F_{2.3}$  lines for anthesis was earlier than the midparental value. Heterosis was evident for all traits with the mean of the  $F_1$  and  $F_{2.3}$  being larger than the midparental averages for PHUN, PEL, PAL, PAW, NN, LL, LW, TN, and smaller for SCI and GDD to anthesis. Earlier maturity for  $F_1$ 's has also been reported in other studies (Kirby and Atkins, 1968; Kambal and Webster, 1966). Given these results and the importance of nonadditive effects in the expression of these traits, dominance and heterosis may be associated in this population.

#### **QTL identification**

Eight out of ten LGs in sorghum were significantly associated with morphological and maturity traits evaluated in this population. The number of QTL per trait, their genetic effects, and linkage map location are presented in Table 3. A total of 42 QTL was identified for all traits, with a range of two to seven QTL per trait. Thirty-eight QTL had LOD score above the threshold level (2.4) and were detected by both methods of analysis (interval mapping and SFAOV) used in this study, the remaining QTL had LOD score values marginally below the threshold (2.1 to 2.3) and were detected only by SFAOV. These findings indicate good agreement between the two methods and are in accordance with results reported previously (Stuber et al., 1992; Pereira and Lee, 1995). Gene action varied from additive or

partial to overdominance, depending on the QTL. Of the total QTL, 45% had partial dominance, 36% complete to overdominance, and 19% additive gene action.

Therefore, nonadditive effects were important in the expression of the morphological traits in this population. In biometrical studies of morphological traits (PH, LL, LW, anthesis, stalk diameter), dominance (Hadley, 1957; Liang and Walter, 1968) and heterosis (Kirby and Atkins, 1968; Kambal and Webster, 1966) have been shown to play important roles. However, the level of dominance has not been defined for any trait, except PHUN. Partial dominance is the mode of gene action reported for PHUN (Hadley, 1957). In our study, only two out of four QTL for PHUN showed partial dominance. However, when all four QTL were fit in a multiple QTL model, their gene action was partial dominance; agreeing with Hadley's findings.

The magnitude of the peak LOD scores related to QTL in this study varied within and among traits. Usually, one or occasionally two QTL per trait had large LOD scores and genetic effects whereas the remaining had smaller scores. Similar patterns have been reported for QTL affecting morphological traits in maize. Of the QTL identified for a trait, only a few accounted for most of the phenotypic variation (Doebley and Stec, 1991 and 1993; Edwards et al., 1992; Veldboom et al., 1994). In this population, QTL explaining more than 26% of the phenotypic variation were observed for all traits except leaf dimensions (LL and LW). An individual QTL explaining the largest phenotypic variation (48%) was located for SCI on LG A, with estimated difference between homozygous parental markers classes of 0.8 cm

(Table 4). This QTL had alleles CK60 for thicker stalks and showed partial dominance. For the components of PH (PHUN, PEL, NN), PHUN has a QTL on LG A with a very large effect, explaining 44% of the phenotypic variation and an estimated difference between parental marker classes of 62 cm (Table 4). Yet, QTL for PHUN explained the largest amount of phenotypic variation (76%) in multiple models in this study. The magnitude of these QTL resembles the effects described for the alleles at the *Dw* loci in sorghum (*dw1*, *dw2*, *dw3*, and *dw4*), which control PHUN (Quinby and Karper, 1954). Complete substitution of recessive for a dominant allele at any of the four *Dw* loci may decrease the stature by 50 cm (House, 1985), which is almost equivalent to the effect of the QTL on LG A. QTL for PHUN have been hypothesized to correspond with the *Dw* loci (Ahnert et al., 1995). Flowering date (anthesis) also has a QTL on LG H explaining 40.8% of the phenotypic variation and an estimated difference between parental marker phenotypic classes of 107 GDD (equivalent difference in flowering date around 5 to 7 days; Table 4). But, its dominance effect was in opposite direction (contributing decreased value of the trait or earlier flowering) with nearly the same magnitude as the additive effect. Components of panicle (PAL, PAW and PEL), and TN each have also a QTL with large effect on LG A. QTL with large effects for anthesis and PH (explaining 85.7% and 54.8% of the phenotypic variation, respectively) were also reported by Lin et al. (1995). In our study, QTL with the largest effects for most of the traits were mapped concentrated in regions on LGs A or H (Fig. 1).

Concentration of QTL in the same region was also observed for PH and anthesis in sorghum (Ahnert et al., 1995; Lin et al., 1995) and in maize (Veldboom et al., 1994) and for several traits in a cross between maize and teosinte (Doebley and Stec, 1991 and 1993). These regions are very important in the expression of these traits and may harbor several QTL clustered that affect each trait, or a QTL for important morphological/physiological trait that has pleiotropic effect on the remaining traits. Details of these associations are described below.

#### **QTL associated with several traits.**

The development of the modern sorghum cultivation system in temperate zones has been based on the manipulation of genes for maturity and PH. Typically, modern varieties have been selected for early maturity to ripe seeds before the first frostings and short stature for mechanized harvesting. Besides these two traits, stronger stalk structure to avoid lodging, and open panicles for rapid grain drying and maturity have also been considered for selection (Poehlman, 1995). Sorghum breeders/geneticists have reported association among these traits and their components (Quinby and Karper, 1954; Kambal and Webster, 1966; Kirby and Atkins, 1968; Esechie et al., 1977). For example, dwarfing genes and genes for earlier maturity shorten plant stature by reducing the number of nodes and the size of the internodes (Doggett, 1988). Herein, we describe the map location and associations among QTL affecting these traits. In many QTL studies, correlated traits are often associated with the same marker loci (Paterson et al., 1991;

Veldboom et al., 1994; Beavis et al., 1994; Lin et al., 1995). In this study, most correlated traits were associated at similar genomic regions suggesting linked and/or pleiotropic effects (Table 2; Fig. 1).

Each component of height (PHUN, PEL, and NN) was significantly associated with four unlinked genomic regions (Table 3). All four QTL located for the main component PHUN coincided with PH QTL on LGs A, E, F, and H. PH and PHUN are highly correlated ( $r = 0.97$ ) and have PI229828 alleles for increased height in all regions. The associations between QTL for PHUN and PH were expected because most of the variation for PH is account for by PHUN (Quinby and Karper, 1954). Indeed, QTL common to PHUN and PH may be explaining the same variation. QTL for the second main component PEL were located on LGs A, B, and G. Two QTL separated by 50 cM were mapped on LG G. Alleles for increased PEL were derived from CK60 and PI229828 on LGs A and B, respectively. Interestingly, these two components of height have one QTL with large effects in opposite direction (with alleles for increased trait coming from different parental origin) coinciding on LG A. Since they were mapped in the same region but in opposite direction, they may be linked in coupling phase. Closely linkage rather than pleiotropy has also been proposed for QTL in common for flowering date and PH in sorghum (Lin et al., 1995). QTL for NN were identified on LGs A, D, F, and H with the taller parent PI229828 contributing alleles for increased NN at three of the regions. PHUN and NN are moderately correlated ( $r = 0.47$ ) and have alleles from



the same parental origin at two QTL closely linked. Overall, this positive association between PHUN and NN was expected because taller plants usually have more nodes in this population.

The larger number of QTL per trait were identified for panicle dimensions (PAL and PAW). Seven QTL on LGs A, B, C, D, E, G, and H were located for PAL with the taller parent PI229828 contributing alleles for increased panicle in six of them (Table 3). However, the QTL (LG A) with alleles from the shorter parent CK60 had the largest effect. Most of the six QTL identified for PAW (LGs A, C, D, F, G, and H) also had alleles PI229828 for increased value. These two traits are highly correlated ( $r = 0.85$ ) and seem to have several QTL in common. The high level of association between these traits should facilitate selection of varieties with desirable "head" characteristics, that is, panicle structures that allow rapid grain drying and maturity. PAL is also a component of PH. Interestingly, low correlations were found between PHUN and PAL ( $r = 0.10$ ) and PHUN and PEL ( $r = -0.14$ ) in this population. However, three QTL were common to PHUN and PAL and one to PHUN and PEL. Correlation between two traits is the net result of the effects of all genes affecting both traits in a positive or negative manner (Falconer, 1989). Therefore, uncorrelated or poorly correlated traits such as these may have QTL in common. On the other hand, these small correlations agree with the relative small pleiotropic or linkage effects reported for some *dw* genes (genes that control PHUN) for panicle components and other traits (Casady, 1965; Graham and Lessman,

1966). Finally, the coincidence of QTL among these traits could be reflecting the pleiotropic effect of some *dw* genes or a cluster of loci with alleles affecting these traits concentrated at the same region.

For Leaf dimensions (LL and LW), four QTL on LGs B, D, F, and H were located for LL with PI229828 alleles contributing for increased leaf size in three of them (Table 3). In contrast, the two QTL identified for LW (LGs A and B) had CK60 alleles for increased trait value. These traits have a low correlation ( $r = 0.20$ ) and only one QTL associated on LG B. Since some dwarfing genes induce the production of broad leaves in maize (Neuffer et al., 1968) and in sorghum (Graham and Lessman, 1966), we were expecting to find associations as the QTL in common between PHUN and LW on LG A and PHUN and LL on LG H. However, PHUN was uncorrelated with LL and LW. Low correlations between PH and LL ( $r = 0.03$ ) and LW ( $r = 0.06$ ) were also reported by Kirby and Atkins (1968).

Stalk circumference was significantly associated with five regions on LGs A, B, C, E, and G with most of the alleles, and the ones with the largest effects coming from CK60 (Table 3). A larger diameter of basal internodes is important for lodging resistance and is associated with shorter PHUN and PEL (Esechie et al., 1977). Also, negative correlation was found between PH and SCI ( $r = -0.87$ ; Kirby and Atkins, 1968). In this present study, SCI was negatively correlated with PHUN and positively with components of panicle (PEL, PAL, and PAW). PHUN and SCI have one overlapping QTL location (LG A) with large effects for both traits but different

parental origins for increased trait values. In contrast, the panicle components and SCI have two overlapping QTL with the same parental origin.

Tiller number was significantly associated with three genomic regions on LGs A, C, and D (Table 3). QTL on LGs C and D were identified only by SFAOV and had smaller effects than QTL on LG A. PI229828 contributed alleles to increase TN in two regions and CK60 in one. Since CK60 has a large stalk and no tillers, and the PI229828 is thinner and tillers, associations such as the negative correlations between TN and SCI ( $r = -0.40$ ) and the positive correlation between TN and PHUN ( $r = 0.24$ ) were expected. These traits have one overlapping QTL with large effect on LG A. In this population, most of the variation in TN was explained by one main QTL on LG A with additive effects.

Flowering date was significantly associated with three regions on LGs E, F, and H with PI229828 contributed the region on LG H with the largest effect (Table 3). This same number of QTL for anthesis were also reported in an  $F_2$  sorghum population (Lin et al., 1995). Sorghum genotypes with delayed flowering typically have more nodes (Doggett, 1988). Therefore, we were expecting to identify association between flowering and components of plant height such as the positive correlation between NN and anthesis ( $r = 0.73$ ). These traits have QTL with a very large effect overlapping on LG H. Also, moderate correlations were observed between anthesis and PHUN ( $r = 0.32$ ), and anthesis and PEL ( $r = -0.39$ ). But, only PHUN and anthesis have one overlapping QTL with large effects on LG H.

High correlations between PH and anthesis ( $r = 0.79$ ) and two QTL in common were also reported by Lin et al. (1995).

Results in this study confirm what has been reported in the sorghum literature about association among important agronomic traits such as components of height, anthesis, SCI, components of panicle, and tillering. Most QTL had their SIs overlap, and around 60% of them were mapped in the 10 cM vicinity indicating association among traits. Overall, selection in one of these traits may result in correlated response in other traits. However, for traits such as panicle components (PAL and PAW) that have high correlations and QTL in common, selection for one trait invariable will result in correlated response in the other trait.

#### **Comparison of QTL in the $F_2$ and $F_{2,3}$ generations**

Several traits were evaluated in the  $F_2$  and  $F_{2,3}$  generations of this population including PH, PAL, PAW, LL, LW, TN, SCI, and GDD. Results of the  $F_2$ - $F_{2,3}$  comparisons have been previously reported for PH (Ahnert et al., 1995). All QTL detected for PH in the  $F_2$  population were located in the same region in both generations, had similar effects and alleles PI229828 for increased height. The  $F_2$  and  $F_{2,3}$  generations were grown at the same location but in 1992 and 1994, respectively, with favorable growing conditions in both years. The planting densities were 45 and 12 cm between plants within rows for the  $F_2$  and  $F_{2,3}$ , respectively. Overall, 25 QTL were identified in the  $F_2$  and 30 in the  $F_{2,3}$  for the traits evaluated in the present study. These results agree with the increased power in QTL detection

proposed for the use of replicated progenies (Cowen, 1988; Soller and Backmann, 1990). Sixteen of these QTL appeared to be detected in both generations (Fig. 1). Typically, these included the QTL with the largest effects in both generations. Trait heritability in the  $F_2$  was positively associated with the number of QTL in common across generations. Overall, traits with higher heritabilities have more QTL in common.

Panicle dimensions (PAL and PAW) had the largest heritability ( $h^2 = 0.57$  and  $0.44$ , respectively in the  $F_2$  population) and number of QTL in common across generations. All six QTL identified for PAL in the  $F_2$  were located at similar regions in the  $F_{2:3}$  generation, except one on LG C. QTL in common in the  $F_2$ - $F_{2:3}$  generations had the largest effects and PI229828 contributing alleles for increased PAL in four of them and CK60 in one (LG A). The QTL unique to  $F_2$  (LG C) had small genetic effects (additive effect = 2.9 cm) and CK60 alleles for increased trait value; and QTL unique to  $F_{2:3}$  (LGs D and E) also had small effects. They were derived from PI229828. The size of their effects may be the main reason they were unique to each generation. Similar results were found for PAW. All five QTL located for this trait in the  $F_2$  were mapped at similar regions in the  $F_{2:3}$ , except on LG C. Also, QTL in common across generations had the largest effects. Panicle components have been used for taxonomic classification in sorghum because they are less affected by the environment (Harlen and deWet, 1972). Data from this study confirm Harlen and deWet's proposition by showing high heritabilities for PAW and

PAL and consistency of QTL across generations.

For leaf dimensions (LW and LL), all three QTL identified for LL in the  $F_2$  were located at similar regions in the  $F_{2.3}$  generation. QTL detected in both generations and environments had the largest effect and PI229828 contributing alleles for increased leaf length. The QTL located only in the  $F_{2.3}$  (LG F) had the smallest effect, which seems to be the main reason it was not detected in the  $F_2$  plants. The QTL located for LW in the  $F_2$  plants (LGs C and H) did not coincide with the map location of QTL in the  $F_{2.3}$  generation. In comparison with the other traits, QTL for LW explained the smallest variation in multiple models in both generations. Yet, LW had the second lowest heritability ( $h^2 = 22\%$ ) in the  $F_2$  generation being highly influenced by the environment.

Tiller number was significantly associated with four regions in the  $F_2$ , but two on (LGs A and C) were located at similar regions in both generations. These QTL were derived from PI229828 and have larger genetic effects in the  $F_2$  generation (additive effects = 0.8 cm). The two QTL identified only in the  $F_2$  (LG B) have also large effects (additive effects = 1.0 cm). In contrast, the QTL identified only in the  $F_{2.3}$  had a very small effect (additive effect = 0.2 cm) and alleles derived from CK60. It is important to note that the  $F_2$  plants were grown at 45 cm between plants within rows, while the  $F_{2.3}$  lines had, on average, 12 cm between plants within rows. The lower density probably permitted a larger number of tillers to develop per plant in the  $F_2$  and likely, accounts to some of the QTL unique to each generation.

Stalk circumference was significantly associated with three regions in the  $F_2$ , but only two (LGs A and C) coincided with QTL for SCI in the present study. QTL detected in both generations had the largest effects with the thicker parent (CK60) contributing alleles for larger stalk circumference. This exemplifies a situation where QTL with large effects were mapped across generations and environments and the ones with small effects were specific to generation and/or environment. Also, two more QTL were mapped in the  $F_{2,3}$  than  $F_2$  generation, possibly due to the more precise estimates of SCI means obtained by replicated progenies.

Flowering date was significantly associated with two regions in the  $F_2$  but only one on LG H coincided at the same map location in the  $F_{2,3}$  generation. This QTL had the largest effect and PI229828 contributing alleles for delayed flowering in both generations. The QTL detected only in the  $F_2$  (LG A) had relatively large genetic effects (additive effects = 45 GDD) and PI229828 alleles for delayed flowering. In contrast, the two QTL detected only in the  $F_{2,3}$  generation had small genetic effects and CK60 alleles for delayed flowering. It appears that the QTL on LG H is the main determinant of flowering date across generations and the remaining might be unique to each environments.

All traits had at least one QTL in common across generations and environments, except LL. Most of the QTL identified in common had additive effects of different magnitudes but in the same direction. In maize, consistency of QTL affecting morphological traits across generations (Austin and Lee, 1995) and

environments (Veldboom and Lee, 1995) have also been reported. In this present study, some differences in QTL mapping in the  $F_2$  and  $F_{2:3}$  populations may be because they were grown in different years, 1992 and 1994, respectively. Also, the  $F_{2:3}$  were grown in a replicated experiment that might have reduced experimental error and increased precision of QTL detection. Nevertheless, the relatively large percentage (64%) of QTL in common indicates those major regions affecting morphological traits in this population were consistent across generations and environments.

#### **Mapping additional genes on the sorghum linkage map**

The assignment of gene loci in a linear order on LGs with the relative genetic distance between them is important for genetic studies, gene cloning, and breeding purposes. For example, the genetic distance between two loci provides the breeder the guidelines for setting up recombinant populations with an appropriate size that allows segregation and isolation of desirable recombinants. In sorghum, around 200 Mendelian genes have been mapped but few LGs have been established (Doggett, 1988). Some of the maturity (*Ma*) and PH (*Dw*) loci previously mapped (Quinby and Karper, 1954; Quinby 1966) have been associated with QTL (Pereira and Lee, 1995; Lin et al., 1995; Ahnert et al., 1995). The maturity *ma1* gene is the most important in the conversion of exotic into temperate zones adapted germplasm and is linked with the dwarf *dw2* gene by 8 cM (Quinby and Karper, 1945). Lin et al. (1995) using an interspecific  $F_2$  population and lines derived from the sorghum



conversion program (exotic, donor, and converted lines) have shown that QTL for PH and anthesis correspond with the mutant loci *dw2* and *ma1*, respectively. An independent study (Ahnert et al., 1995) used an  $F_{2:3}$  population to assign QTL for PH and anthesis to the *dw2* and *ma1* loci, respectively. Pereira and Lee (1995) and Ahnert et al. (1995) have associated QTL for PH with the *dw2*, *dw3*, and *dw4* loci. Besides these QTL, many more were mapped for the ten morphological traits analyzed in this study, extending the number of genes mapped in sorghum. As stated by Veldboom et al. (1994), QTL may represent a more "crude" or generalized approach to define regions associated with the expression of traits compared with the conventional mapping by using mutants. Nevertheless, they are of great importance for sorghum in which few linkages have been established and not many genes mapped compared to maize.

Regions in sorghum concentrating major QTL (LGs A and H) in this study and in (Ahnert et al., 1995; Lin et al., 1995) may represent the most preeminent target for map-based cloning (Tanksley et al., 1995) and detailed molecular and functional studies. Better knowledge of the significance of these regions may be useful for comparative mapping and gene transfer endeavors. Some of these regions may be orthologous with maize (Pereira and Lee, 1995; Lin et al., 1995; Ahnert et al., 1995) and harbor loci for PH, flowering date and other morphological traits important for genetic improvement of these species and other members of the *Gramineae* family. Important homoeologous regions between sorghum and maize could be cloned in

one species and used for map-based cloning in that species or in the homoeologous counterpart species (Tanksley et al., 1995). Finally, mapping important agronomic/morphological traits may facilitate comparative mapping, cloning of QTL, and genetic and evolutionary studies.

### References

- Ahnert, D., Lee, M., and Pereira, M.G., 1995. Identification of plant height QTL in sorghum across generations and comparative mapping with maize. (In preparation).
- Austin, D.F. and Lee, M., 1995. Mapping QTL for morphological traits in a recombinant inbred maize population. (Accepted in Theor. Appl. Genet.)
- Beavis, W.D., Grant, D., Albertsen, M., and Fincher, R., 1991. Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. Theor. Appl. Genet., 83:141-145.
- Beavis, W.D., Smith, O.S., Grant, D., and Fincher, R., 1994. Identification of quantitative trait loci using a small sample of topcrossed and F<sub>4</sub> progeny from maize. Crop Sci. 34:882-896.
- Berhan, A.M., Hulbert, S.H., Butler, L.G., and Bennetzen, J.L., 1993. Structure and evolution of the genomes of *Sorghum bicolor* and *Zea mays*. Theor. Appl. Genet., 86:598-604.
- Binelli, G., Gianfranceschi, L., Pe, M.E., Taramino, G., Busso, C., Stenhouse, J., and Ottaviano, E., 1992. Similarity of maize and sorghum genomes as revealed by maize RFLP probes. Theor. Appl. Genet., 84:10-16.
- Casady A.J., 1965. Effect of single height (Dw) gene of sorghum on grain yield, grain yield components, and test weight. Crop Sci., 5:385-389.
- Chittenden, L.M., Schertz, K.F., Lin, Y.R., Wing, R.A., and Paterson, A.H., 1994. A detailed RFLP map of *Sorghum bicolor* X *S. Propinquum*, suitable for high-density mapping, suggests ancestral duplication of Sorghum chromosomes or chromosomal segments. Theor. Appl. Genet., 87:925-933.

- Cochran, W.G., and Cox, G.M., 1957. Experimental designs. John Wiley & Sons, N.Y.
- Cowen, N.M., 1988. The use of replicated progenies in marker-based mapping of QTLs. *Theor. Appl. Genet.*, 75:857-862.
- Doebley, J., and Stec, A., 1991. Genetic analysis of the morphological differences between maize and teosinte. *Genetics*, 129:285-295.
- Doebley, J., and Stec, A., 1993. Inheritance of morphological differences between maize and teosinte: comparison of results for two  $F_2$  populations. *Genetics*, 134:559-570.
- Doggett, H., 1988. Sorghum. Longman Group U.K. Ltd., Essex, England
- Duncan, R., Bramel-Cox, P.J., and Miller, F.R., 1991. Contributions of introduced sorghum germplasm to hybrid development in the USA. (CSSA special publication no. 17:69-101).
- Edwards, M.D., Helentjaris, T., Wright, S., and Stuber, C.W., 1992. Molecular-marker-facilitated investigations of quantitative trait loci in maize. *Theor. Appl. Genet.*, 83:765-774.
- Edwards, M.D., Stuber, C.W., and Wendel, J.F., 1987. Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. *Genetics*, 116:113-125.
- Esechie, H.A., Maranville, J.W., and Ross, W.M., 1977. Relationship of stalk morphology and chemical composition to lodging resistance in sorghum. *Crop Sci.*, 17:609-612.
- Falconer, D.S. 1989. Introduction to quantitative genetics. 3rd ed. John Wiley & Sons, Inc., NY.
- Graham, D., and Lessman, K.J., 1966. Effects of height on yield and yield components of two isogenic lines of *Sorghum vulgare* Pers. *Crop Sci.*, 6:372-374.
- Hammer, G.L., and Vanderlip, R.L., 1989. Genotype-by-environment interaction in grain sorghum. III. Modeling the impact in field environments. *Crop Sci.* 29:385-391.

- Hadley, H.H., 1957. An analysis of variation in height in sorghum. *Agron. Jour.*, 49:144-147.
- Hallauer, A.R., and Miranda, J.B., 1988. Quantitative genetics in maize breeding 2nd edn., Iowa State University Press, Ames, Iowa.
- Harlen, J.R., and deWet, J.M.J., 1972. A simplified classification of cultivated sorghum. *Crop Sci.*, 12:172-176.
- House, L.R., 1985. A guide to sorghum breeding. Second edition. ICRISAT, Patancheru, India.
- Hulbert, S.H. Richter, T.E. Axtell, J.D., and Benetzen, J.L., 1990. Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. *Proc. Natl. Acad. Sci. U.S.A.*, 87:4251-4255.
- Lander, E.S., and Botstein, D., 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, 121:185-199.
- Lin, Y.R., Schertz, K.F., and Paterson, A. H., 1995. Comparative analysis of QTLs affecting plant height and maturity across the *Poaceae*, in reference to an interspecific sorghum population. *Genetics*, 141:391-411.
- Liang, G.H.L. and Walter, T.L., 1968. Heritability estimates and gene effects for agronomic traits in grain sorghum, *Sorghum vulgare* Pers. *Crop Sci.* 8:77-81.
- Jansen, R.C., and Stam, P., 1994. High resolution of quantitative traits into multiple loci via interval mapping. *Genetics*, 136:1447-1455.
- Kambal, A.E. and Webster, O.J., 1966. Manifestations of hybrid vigor in grain sorghum and the relations among the components of yield, weight per bushel, and height. *Crop Sci.* 6:513-515.
- Kirby, J.S. and Atkins, R.E., 1968. Heterotic response for vegetative and mature plant characters in grain sorghum, *Sorghum bicolor* (L.) Moench. *Crop Sci.* 8:335-339.
- Mather, K., and Jinks, J.L., 1971. Biometrical genetics. Chapman and Hall, London.

- Miller, F.R., 1982. Genetics and Environmental response characteristics of sorghum. In: House, L.R., Munghogho, L.K. and Peacock, J.M. (eds). Sorghum in the Eighties. ICRISAT, Patancheru, India, pp. 393-402.
- Neuffer, M.G., Jones, L., and Zuber, M.S., 1968. The mutants of maize. Crop Sci. Soc. of Amer. Madison, Wisconsin.
- Paterson, A.H., Damon, S., Hewitt, J.D., Zamir, D., Rabinowitch, H.D., Lincoln, S.E., Lander, E.S., and Tanksley, S.D. 1991. Mendelian factors underlying quantitative traits in tomato: Comparison across species, generations and environments. Genetics, 127:181-197.
- Pereira, M.G., Lee, M., Bramel-Cox, P., Woodman, W., Doebley, J., and Whitkus, R., 1994. Construction of an RFLP map in sorghum and comparative mapping in maize. Genome, 37:236-243.
- Pereira, M.G., 1993. Restriction fragment length polymorphism in sorghum (*Sorghum bicolor* (L.) Moench): genetic map construction and analysis of quantitative traits. Ph.D. dissertation. Iowa State University, Ames, Iowa.
- Pereira, M.G., and Lee, M., 1995. Identification of genomic regions affecting plant height in sorghum and maize. Theor. Appl. Genet., 90:380-388.
- Pereira, M.G., Ahnert, D., Lee, M., and Klier, K. 1995. Genetic mapping of quantitative trait loci for panicle characteristics and seed weight in sorghum. Rev. Brasil. Genet., 18:249-257.
- Poehlman, J.M., 1987. Breeding sorghum and millet. In: breeding field crops. AVI Pub. Co., Westport, Connecticut. pp. 308-358.
- Poehlman, J.M., and Sleper, D.A., 1995. Breeding sorghum. In: breeding field crops. 4th ed. Iowa State University Press/Ames, Iowa. pp. 345-366.
- Quinby, J.R. and Karper, R.E., 1945. The inheritance of three genes that influence time of floral initiation and maturity date in Milo. J. Am. Soc. Agron., 37:919-936.
- Quinby, J.R. and Karper, R.E., 1954. Inheritance of height in sorghum. Agron. J., 46:211-216.
- Quinby, J.R., 1966. Fourth maturity gene locus in sorghum. Crop Sci., 6:516-518.

- Quinby, J.R., 1972. Influence of maturity genes on plant growth in sorghum. *Crop Sci.*, 12:490-492.
- Quinby, J.R., 1975. The genetics of sorghum improvement. *J. Hered.*, 66:56-62.
- SAS Institute., 1988. SAS introductory guide for personal computers. SAS Institute, Inc., Cary, North Caroline.
- Schön, C.C., Melchinger A.E., Boppenmaier, J., Brunklaus-Jung, E., Herrmann, R.G., and Seitzer, J.F., 1994. RFLP mapping in maize: quantitative trait loci affecting testcross performance of elite european flint lines. *Crop Sci.*, 34:378-389.
- Searle, S.R. (1971). *Linear models*. John Wiley and Sons, New York.
- Shapiro, S.S. and Wilk, M.B., 1965. An analysis of variance for normality (complete samples). *Biometrika*, 52:591-611.
- Snowden, J.D., 1936. *The cultivated races of sorghum*. Allard and Son, London.
- Stuber, C.W., Edwards, M.D., Wendel, J.F., 1987. Molecular marker-facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component traits. *Crop Sci.*, 27:639-648.
- Stuber, C.W., Lincoln, S.E., Wolff, D.W., Helentjaris, T., and Lander E.S., 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics*, 132:823-839.
- Soller, M. and Beckmann, J.S., 1990. Marker-based mapping of quantitative trait loci using replicated progenies. *Theor. Appl. Genet.*, 80:205-208.
- Tanksley, S.T., Ganai, M.W., and Martin G.B., 1995. Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. *Trends in Genetics*, 11:63-68
- Veldboom, L.R., Lee, M., and Woodman, W. L., 1994. Molecular marker-facilitated studies in an elite maize population: I. Linkage analysis and determination of QTL for morphological traits. *Theor. Appl. Genet.*, 88:7-16.
- Veldboom, L.R. and Lee, M., 1995. Genetic mapping of morphological traits in maize in stress and nonstress environments. *Crop Sci.* (In review).

- Whitkus, R., Doebley, J., and Lee, M., 1992. Comparative genome mapping of sorghum and maize. *Genetics*, 132:1119-1130.
- Xu, W., Crasta, O., Rosenow, D., Mullet, J., and Nguyen, H., 1994. Major QTLs for post-flowering drought resistance in grain sorghum. *International Plant Genome III Conference*, 1995.





Traits					
PEL	LL	LW	SCI	TN	GDD
cm	cm	cm	cm	number	GDD
51	67	6.8	7.0	0.1	1224
69	83	5.6	4.4	2.4	1219
74	79	6.7	5.1	1.4	1157
68	77	6.6	5.0	1.3	1188
47 - 87	63 - 88	5.4 - 7.8	4.0 - 7.0	0.0 - 2.8	1045 - 1360
53 ± 7.3**	15 ± 2.1**	0.19 ± 0.02**	0.17 ± 0.02**	0.17 ± 0.03**	3379 ± 422**
19.6 ± 2.4	5.5 ± 0.6	0.09 ± 0.01	0.08 ± 0.01	0.22 ± 0.02	595 ± 72.9
152 ± 132**	126 ± 104**	0.6 ± 0.5**	3.7 ± 2.8**	2.3 ± 2**	288.6 ± 7.5 <sup>ns</sup>
84	84	81	81	60	92
0.79 - 0.88	0.80 - 0.80	0.74 - 0.85	0.75 - 0.85	0.47 - 0.69	0.89 - 0.93
—	32 ± 3	22 ± 4	30 ± 3	8 ± 2	37 ± 3

\*\* Significant at 0.01 probability level.

<sup>ns</sup> Non significant at 0.01 probability level.

--- Traits measured only in the F<sub>23</sub> population.

Table 2. Phenotypic correlations among morphological and maturity traits for 152 F<sub>2:3</sub> sorghum lines.

	PHUN	PEL	PAL	NN	PAW	LL	LW	TN	SCI	GDD
PH	0.97**	0.07	0.21**	0.42**	0.02	0.11	0.00	0.22**	-0.35**	0.23**
PHUN		-0.14	0.10	0.47**	-0.07	0.04	-0.00	0.24**	-0.40**	0.32**
PEL			0.50**	-0.26**	0.46**	0.27**	0.09	-0.11	0.22**	-0.39**
PAL				0.25**	0.85**	0.30**	0.10	-0.11	0.40**	0.06
NN					0.13	-0.0	-0.07	-0.02	0.15	0.73**
PAW						0.30**	0.11	-0.16*	0.45**	0.00
LL							-0.20**	0.20**	-0.03	0.19**
LW								-0.24**	0.32**	-0.15
TN									-0.40**	0.02
SCI										0.13

\*, \*\* Significant at the 0.05 and 0.01 probability levels, respectively.  
Trait acronyms are indicated in the Materials and Methods section.

Table 3. Genetic locations, percentage of phenotypic variation, and genetic effects of QTL detected in 152 F<sub>23</sub> sorghum lines of the population CK60 versus PI229828.

Linkage group	Interval†	Distance‡ (cM)	Max. LOD¶ score	%5²p	Genetic effects§			Gene action	Direction§
					a	d	d/a		
Plant height o the uppermost node (cm)									
A	isu123-isu116	20.9	13.6	41.4	33.8	44.0	1.3	OD	PI229828
E	isu140-pio10016	19.0	5.4	19.3	22.0	12.6	0.5	PD	PI229828
F	isu071-isu130	26.1	2.5	11.1	7.8	9.0	1.1	D	PI229828
H	isu032B-isu156	13.2	5.8	18.9	21.7	-8.2	-0.3	PD	PI229828
Total			35.9	76					
Peduncle length (cm)									
A	isu123-isu116	20.9	9.2	30.1	-5.5	-2.8	0.5	PD	CK60
B	umc71-isu058	5.1	5.3	18	3.8	5.2	1.3	OD	PI229828
G	isu068-bnl06.16	31.8	4.3	12.2	2.0	4.4	2.2	OD	PI229828
G	npi426-isu057	6.0	2.9	9.5	3.1	0.42	0.1	A	PI229828
Total			21.7	53.6					

† Flanking markers of the most likely QTL position.

‡ Genetic distance measured in cM between flanking markers.

¶ LOD threshold = 2.4.

§ Additive effects are associated with the allele from PI229828. Thus, a negative value means that the PI229828 allele decreases trait value.

§ Direction of response is the parent whose additive value of a marker allele increased trait value.

Table 3. (Continued)

Linkage group	Interval†	Distance‡ (cM)	Max. LOD score¶	%δ²p	Genetic effects §			Gene action	Direction§
					a	d	d/a		
Panicle length (cm)									
A	isu123-isu116	20.9	7.8	27.7	-2.5	-0.04	0.0	A	CK60
B	umc71-isu058	5.1	2.9	8.9	0.9	0.16	0.1	A	PI229828
C	isu119-isu078A	8.5	8.0	24.6	1.9	1.04	0.5	PD	PI229828
D	bnl05.40-pio20566	14.0	2.4	7.8	1.0	-0.34	-0.3	PD	PI229828
E	isu140-pio10016	19.0	3.2	9.3	1.3	0.78	0.6	PD	PI229828
G	isu068-bnl06.16	31.8	3.9	11.3	1.0	0.56	0.5	PD	PI229828
H	isu032B-isu156	13.2	4.4	14.6	1.9	0.78	0.4	PD	PI229828
Total			33.2	69.6					
Tiller number (number)									
A	umc33-isu123	12.4	8.7	26.6	0.38	0.08	0.2	A	PI229828
C	isu092-isu070	16.3	2.3	6.9	0.16	0.1	0.6	PD	PI229828
D	isu080-isu056	30.6	2.3	11.0	-0.20	-0.26	1.3	OD	CK60
Total			13.2	37.8					
Panicle width (cm)									
A	isu123-isu116	20.9	5.9	21.1	-3.0	0.48	-0.1	A	CK60
C	isu119-isu078A	8.5	9.6	27.9	3.1	0.38	0.1	A	PI229828
D	isu042-npi104A	34.8	2.5	11.0	2.0	1.3	0.6	PD	PI229828
F	isu130-npi400	9.5	2.6	8.0	-1.5	0.68	-0.4	PD	CK60
G	isu021-isu068	9.7	4.8	13.4	1.3	-0.50	-0.3	PD	PI229828
H	pio10005-isu032B	8.0	4.1	14.4	1.9	1.9	1.0	D	PI229828
Total			32.5	70.6					

Table 3. (Continued)

Linkage group	Interval†	Distance‡ (cM)	Max. LOD score¶	%δ²p	Genetic effects‡			Gene action	Direction§
					a	d	d/a		
Number of nodes (number)									
A	isu116-isu074	15.5	4.0	16.6	0.20	1.2	6.0	OD	PI229828
D	pio20608-isu107	12.4	2.9	9.4	0.24	-0.14	-0.5	PD	PI229828
F	isu037-isu117	3.9	2.8	8.4	-0.36	-0.14	0.3	PD	CK60
H	isu032B-isu116	13.2	11.5	32.9	0.73	0.06	0.0	A	PI229828
Total			22.7	58.6					
Leaf length (cm)									
B	isu058-isu030	2.5	6.0	17.1	2.3	0.90	0.4	PD	PI229828
D	isu126A-isu042	26	3.2	10.6	1.5	-1.9	-1.2	D	PI229828
F	isu151-isu071	10.4	2.1	6.9	-1.3	1.1	-0.8	PD	CK60
H	isu156-isu034	8.6	6.1	18.7	2.2	-2.0	0.9	D	PI229828
Total			18.1	45.0					
Leaf Length (cm)									
A	isu095-umc33	8.0	5.5	15.5	-0.21	0.26	-1.2	D	CK60
B	isu138-isu126B	28.1	3.5	15.0	-0.21	0.20	-0.9	D	CK60
Total			8.1	24.4					

Table 3. (continued)

Linkage group	Interval†	Distance‡ (cM)	Max. LOD score¶	%δ²p	Genetic effects §			Gene action	Direction§
					a	d	d/a		
Flowering date (GDD)									
E	isu026-umc64	28.7	2.8	8.2	-14.2	-30.4	2.1	OD	
F	isu037-isu117	3.9	2.2	6.7	-23.1	-9.0	0.3	PD	
H	isu032B-isu156	13.2	13.9	40.8	53.2	-50.8	-0.9	D	
Total			19.4	51.2					
Stalk circumference (cm)									
A	isu123-isu116	20.9	16.6	47.8	-0.45	-0.40	0.80	PD	CK60
B	isu147-isu155	22.5	2.7	15.8	-0.17	-0.00	0.00	A	CK60
C	isu027-npi209	22.9	4.9	18.4	-0.14	-0.32	2.20	OD	CK60
E	pio10016-pio20714	27.9	2.5	9.2	-0.14	0.08	-0.60	PD	CK60
G	isu068-bnl06.16	31.8	2.7	8.1	0.06	0.20	3.3	OD	PI229828
Total				29.3	68.0				

Table 4. Genotypic class averages of QTL affecting morphological and maturity traits for 152  $F_{2:3}$  sorghum lines.

Linkage group	Nearest† loci	Genotypic Classes‡		
		CK/CK(μAA)	CK/PI(μAB)	PI/PI(μBB)
Plant height to the uppermost node (cm)				
A	isu116	172.2	222.4	234.5
E	isu140	198.2	224.0	227.0
F	isu130	200.8	221.3	224.4
H	isu032B	206.9	217.5	253.7
Peduncle length (cm)				
A	isu116	76.6	68.0	64.5
B	isu058	61.8	70.0	69.2
G	isu068	63.0	69.7	70.6
G	isu057	64.6	68.6	70.7
Panicle length (cm)				
A	isu123	31.4	29.0	26.5
B	umc71	26.3	29.3	28.3
C	isu119	27.0	28.5	30.6
D	pio20566	27.6	28.1	30.3
E	isu140	26.4	28.7	30.3
G	isu068	26.4	28.8	29.8
H	isu032B	26.4	30.6	29.3
Number of nodes (number)				
A	isu116	10.6	11.2	10.8
D	pio20608	10.7	10.9	11.4
F	isu037	11.3	11.1	10.6
H	isu032B	10.3	11.0	11.9

† Nearest marker of the most likely QTL position.

‡ Trait means for homozygous CK60, heterozygous CK60/PI229828, and homozygous PI229828 alleles at QTL affecting height.

Table 4. (Continued)

Linkage	Nearest†	Genotypic Classes‡		
group	loci	CK/CK(μAA)	CK/PI(μAB)	PI/PI(μBB)
Panicle width (cm)				
A	isu123	30.7	28.5	25.5
C	isu119	23.6	28.1	31.2
D	isu042	26.2	27.6	29.8
F	npi400	30.0	28.0	26.0
G	isu068	24.6	28.0	29.7
H	pio10005	25.6	28.5	29.5
Leaf length (cm)				
B	isu058	73.9	77.1	79.1
D	isu126A	75.3	76.3	79.0
F	isu151	77.5	77.7	75.0
H	isu156	74.9	76.7	80.2
Leaf width (cm)				
A	isu033	6.8	6.7	6.3
B	isu138	6.7	6.6	6.3
Number of tillers (number)				
A	umc33	0.8	1.2	1.6
C	isu092	1.0	1.3	1.5
D	isu080	1.5	1.2	1.2
Stalk circumference (cm)				
A	isu123	5.6	5.0	4.8
B	umc71	5.2	5.1	4.9
C	isu027	5.4	5.0	4.9
E	pio20714	5.3	5.0	4.9
G	isu068	4.8	5.1	5.1
Anthesis (GDD)				
E	isu026	1217.2	1175.3	1181.1
F	isu037	1206.7	1194.4	1164.1
H	isu032B	1151.2	1184.0	1258.8



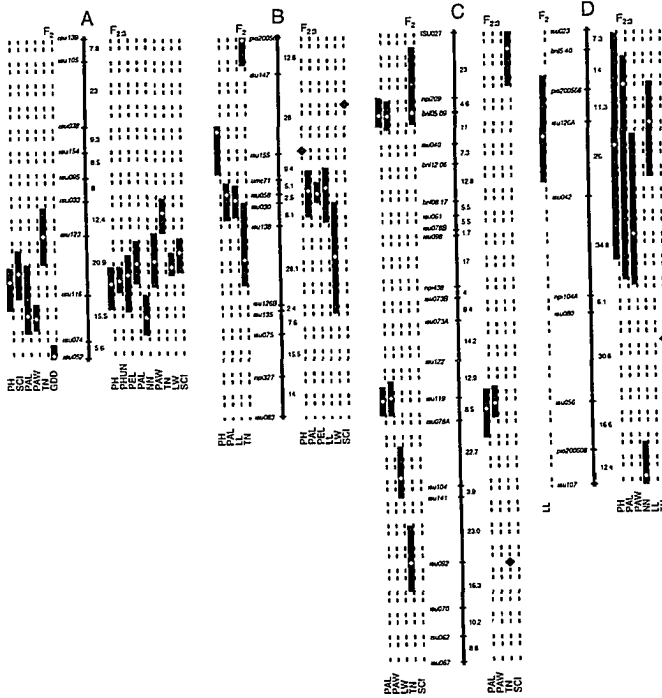
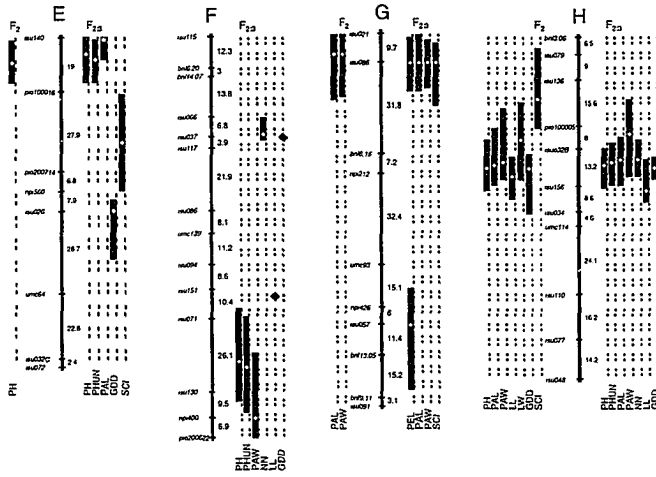


Figure 1. Linkage group location of QTL affecting morphological and maturity traits in the  $F_2$  and their  $F_{2.3}$  progenies. QTL positions are indicated by the white diamond with their one-LOD (black bar) support intervals. Black diamonds (◆) indicate QTL with LOD score below threshold level (2.4), located only by single-factor analysis of variances. Trait acronyms are indicated in the Materials and Methods section. Traits as PHUN, NN, and PEL were only recorded in the  $F_{2.3}$  generation. All other traits were recorded in  $F_2$  and  $F_{2.3}$  generations.



### GENERAL CONCLUSIONS

A survey of genetic diversity in a set of 47 B- and 58 R- elite sorghum inbred lines and characterization of quantitative variation for morphological traits in 152  $F_{2,3}$  lines allowed the investigation of important issues in sorghum genetics. The R- and B-lines set were examined for the level of genetic diversity and relationships based on RFLPs and coancestry coefficients ( $f$ ). The  $F_{2,3}$  lines were used for QTL mapping of morphological traits, comparison of results obtained in the  $F_{2,3}$  to those obtained previously in the  $F_2$  generation, comparison of QTL map location for plant height in sorghum and maize, and association between QTL affecting plant height and qualitative *Dw* loci which control height in sorghum.

Our findings support the sorghum breeders' popular belief that A- and B-lines have less genetic diversity than R-lines based on RFLPs. On average, any two B-lines had 76% of the RFLP bands in common, while two R-lines had 67% of the bands in common. Reduced variability among B-lines might be due to the wide use of Kafir nuclear factors and Milo cytoplasm for the development of male-sterile. The level of genetic diversity detected in R- and B-lines in our study was greater than that obtained in sorghum by using RAPDs (Tao et al., 1993) but was similar to earlier results from RFLPs (Vierling et al., 1994). However, diversity was less than that reported in maize inbred lines (Smith et al., 1990; Melchinger et al., 1991; Boppenmaier et al., 1992). Similarity estimates defined by  $f$  were low for both R- and B-lines. For many pairs of lines  $f$  values were zero(0). The  $f$  estimates are not

in agreement with previous reports in sorghum, which suggest a higher level of genetic similarity among B-lines (Poehlman, 1987; Duncan et al., 1991). These findings could be due to either the absence of a detailed pedigree record in sorghum or the use of several simplifying assumptions to calculate  $f$  that may not be entirely accurate for elite sorghum inbred lines.

Genetic similarity based on RFLPs and  $f$  can be used as alternative measures of genetic relationships among genotypes. The regression of genetic similarity on  $f$  examined the relationships between these two similarity estimates. Although the correlations ( $r = 0.5$ ) between these two similarity measures were not high, they were positive and significant. These results indicate that the RFLP data may help quantify the degree of relatedness in elite sorghum germplasm.

Cluster analysis of genetic similarity estimates grouped the inbred lines in accordance with parental types, pedigree records, and the classification system used by breeders. With a few exceptions, R- and B-lines were separated into distinct groups suggesting that their difference at the DNA level could be assessed by the RFLP procedure used in this study. The R-lines were grouped in two main clusters. One composed of early introductions, mainly Feterita germplasm. The other of more recent introductions, mainly Zera-zera and Yellow source germplasm. The associations among germplasm groups revealed by principal component analysis (PCA) agreed with the results obtained by cluster analysis. The R-lines were widely spread all over the plot forming three main loose clusters. In contrast,

most of the B-lines formed one tight cluster.

Eight out of ten linkage groups in sorghum were significantly associated with the morphological traits evaluated in the  $F_{2:3}$  population. The number of QTL per trait ranged from two to seven, but three to four were identified for most traits. The magnitude of the peak LOD scores related to these QTL varied within and among traits. Usually, one or occasionally two QTL per trait had large LOD scores and genetic effects whereas the remaining had smaller scores. A similar pattern has been reported for QTL affecting morphological traits in maize (Doebley and Stec, 1991 and 1993; Edwards et al., 1992; Veldboom et al., 1994). In this population, QTL explaining more than 26% of the phenotypic variation were observed for all traits, except leaf dimensions.

Gene action varied from additive or partial to overdominance, depending on the QTL. However, most QTL showed partial to overdominance gene action indicating that non-additive effects are very important in the expression of morphological traits in this population. Given that the parents used to form the  $F_{2:3}$  population diverged in most traits, the alleles that contributed to increase traits values were usually predicted in accordance with the phenotype of the parent. For example, PI229828 had larger means for plant height components. Most of the alleles increasing these traits came from this parent.

Larger numbers of QTL per trait were identified in the  $F_{2:3}$  than in the  $F_2$  generation. These results agreed with the increased power in QTL detection

proposed for the use replicated progenies (Cowen, 1988; Soller and Beckmann, 1990). Around 69% of the QTL mapped in the  $F_2$  plants coincided at similar regions in the  $F_{2:3}$  generation. QTL with the largest effects were usually consistent across generations and environments. Yet, the additive effects of QTL in common regions in the  $F_2$  and  $F_{2:3}$  generations were of different magnitudes but in the same direction.

Overall, correlated traits in the  $F_{2:3}$  population had QTL coinciding at similar regions indicating linked and/or pleiotropic effects. For example, panicle length and panicle width were highly correlated and had most QTL at similar regions with alleles for increased trait value from the same parental origin.

Plant height QTL on linkage groups A, E, F, and H may correspond with the qualitative *Dw* loci which control most of the variation in height. Also, a QTL for anthesis located on linkage group H may correspond with the maturity *ma1* loci. These QTL may be associated with the maturity and dwarf genes that are being introgressed to convert tropical germplasm to temperate regions. This information could be used in marker-assisted backcross breeding programs to speed up the sorghum conversion process.

Comparisons of QTL map location in sorghum and maize have allowed the identification of QTL affecting plant height in regions defined by common RFLP loci between these species. Such correspondences suggest that genes affecting height in sorghum and maize may be orthologous.

## GENERAL REFERENCES

- Aldrich, P.R., and Doebley, J. 1992. Restriction fragment variation in the nuclear and chloroplast genomes of cultivated and wild *Sorghum bicolor*. Theor. Appl. Genet. 85:293-302.
- Aldrich, P.R., Doebley, J., Schertz, K. F., and Stec, A. 1992. Patterns of allozyme variation in cultivated and wild *Sorghum bicolor*. Theor. Appl. Genet. 85:451-460.
- Boppenmaier, J., Melchinger, A.E., Brunklaus-Jung, E., Geiger, H.H., and Herman, R.G. 1992. Genetic diversity for RFLPs in European maize Inbreds: I. Relation to performance of flint x dent crosses for forage traits. Crop Sci. 32:895-902.
- Burr, B., Evola, S.V., Burr, F.A., and Beckman, J.S., 1983. The application of restriction fragment length polymorphisms to plant breeding. p. 45-69. In Genetic engineering principles and methods Vol. 5. Edited by J.K. Setlow and A. Highlander. Plenum, New York.
- Cowen, N.M., 1988. The use of replicated progenies in marker-based mapping of QTLs. Theor. Appl. Genet., 75:857-862.
- Doggett, H. 1988. Sorghum. Longmans, Green & Co., London.
- Doebley, J., and Stec, A., 1991. Genetic analysis of the morphological differences between maize and teosinte. Genetics, 129:285-295.
- Doebley, J., and Stec, A., 1993. Inheritance of morphological differences between maize and teosinte: comparison of results for two F<sub>2</sub> populations. Genetics, 134:559-570.
- Duncan, R.R., Bramel-Cox, P.J., and Miller, F.R. 1991. Contributions of introduced sorghum germplasm to hybrid development in the USA. CSSA special publication No. 17. pp. 69-101.
- Edwards, M.D., Helentjaris, T., Wright, S., and Stuber, C.W., 1992. Molecular-marker-facilitated investigations of quantitative trait loci in maize. Theor. Appl. Genet., 83:765-774.
- House, L.R., 1985. A guide to sorghum breeding. Second edition. ICRISAT, Patancheru, India.

- Hulbert, S.H., Richter, T.E., Axtell, J.D., and Bennetzen, J.L. 1990. Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. *Proc. Natl. Acad. Sci. (USA)*. 87: 4251-4255.
- Klier, K.E., 1988. Relationship of shattercane to cultivated and feral *Sorghum* in the midwestern United States. Ph.D. dissertation. Iowa State University, Ames, Iowa.
- Kramer, N.W. 1987. Grain sorghum production and breeding Historical perspectives to future prospects. *Proc. Annu. Corn. and Sorghum Res. Conf.* 42:1-19.
- Lin, Y.R., Schertz, K.F., and Paterson, A. H., 1995. Comparative analysis of QTLs affecting plant height and maturity across the *Poaceae*, in reference to an interspecific sorghum population. *Genetics*, 141:391-411.
- Melchinger, A.E., Graner, A., Singh, M., and Messmer, M.M. 1994. Relationships among European barley germplasm: I. Genetic diversity among winter and spring cultivars revealed by RFLPs. *Crop Sci.* 34:1191-1199.
- Miller, F.R. 1979. The breeding of sorghum. p. 128-136. In M. K. Harris (ed) *Biology and breeding for resistance to arthropods & pathogens in agricultural plants*. Texas A & M Univ., Texas Agric. Exp. Stn. MP-1451.
- Morden, C. W., J. Doebley, and K. F. Schertz. 1990. Allozyme variation among the spontaneous species of *Sorghum* section *Sorghum* (Poaceae). *Theor. Appl. Genet.* 80: 296-304.
- Murray, M.G., Ma.Y., Severson, J.R., West, D.P., and Kramer, G.R., 1988. Restriction fragment length polymorphism: what are they and how can breeders use them? p. 73-87. In 43rd Proc. Annu. Corn & Sorghum Res. Conference.
- Pereira, M.G., Lee, M., Bramel-Cox, P., Woodman, W.L., Doebley, J., and Whitkus, R. 1994. Construction of an RFLP map in sorghum and comparative mapping in maize. *Genome* 37: 236-243.
- Pereira, M.G. 1993. Restriction fragment length polymorphism in sorghum (*Sorghum bicolor* (L.) Mince): genetic map construction and analysis of quantitative traits. Ph.D. dissertation. Iowa State University, Ames, Iowa.
- Pereira, M.G., and Lee, M., 1995. Identification of genomic regions affecting plant height in sorghum and maize. *Theor. Appl. Genet.*, 90:380-388.



- Pereira, M.G., Ahnert, D., Lee, M., and Klier, K. 1995. Genetic mapping of quantitative trait loci for panicle characteristics and seed weight in sorghum. *Rev. Brasil. Genet.*, 18:249-257.
- Poehlman, J.M. 1987. Breeding sorghum and millet. p. 508-558. In *Breeding field crops*. AVI Pub Co., Westport, Connecticut.
- Quinby, J.R., and Karper, R.E., 1945. The inheritance of three genes that influence time of flower initiation and maturity date in sorghum. *Jour. Amer. Soc. Agron.* 37:916-936.
- Quinby, J.R., and Karper, R.E., 1954. Inheritance of height in sorghum. *Agron. J.*, 46:211-216.
- Quinby, J.R., 1966. Fourth maturity gene locus in sorghum. *Crop Sci.* 6:516-518.
- Quinby, J.R., 1967. The maturity genes of sorghum. *Advanc. Agron. J.* 46:211-216.
- Quinby, J.R., 1975. The genetics of sorghum improvement. *J. Hered.* 66:56-62.
- Smith, O.S., Smith, J.S.C., Bowen, S.L., Tenborg, R.A., and Wall, S.J. 1990. Similarities among a group of elite maize inbreds as measured by pedigree,  $F_1$  grain yield, heterosis, and RFLPs. *Theor. Appl. Genet.* 80:833-840.
- Soller, M. and Beckmann, J.S., 1990. Marker-based mapping of quantitative trait loci using replicated progenies. *Theor. Appl. Genet.*, 80:205-208.
- Tao, Y., Manners, J.M., Ludlow, M.M., and Henzel, R.G. 1993. DNA polymorphisms in grain sorghum (*Sorghum bicolor* (L.) Mince). *Theor. Appl. Genet.* 86: 679-688.
- Tanksley, S.D., 1983. Molecular markers in plant breeding. *Plant Mol. Biol. Rept.* 1:3-8.
- Veldboom, L.R., Lee, M., and Woodman, W.L. 1994. Molecular marker-facilitating studies in an elite maize population: I. Linkage analysis and determination of QTL for morphological traits. *Theor. Appl. Genet.* 88: 7-16.

- Vierling, R.A., Xiang, Z., Joshi, C.P., Gilbert, M.L., and Nguyen, H.T. 1994. Genetic diversity among elite sorghum lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs. *Theor. Appl. Genet.* 87:816-820.
- Webster, O.J., 1976. Sorghum vulnerability and germplasm resources. *Crop Sci.* 16:553-557.
- Whitkus, R., Doebley, J., and Lee, M. 1992. Comparative genome mapping of sorghum and maize. *Genetics* 132: 1119-1130.
- Xu, W., Crasta, O., Rosenow, D., Mullet, J., and Nguyen, H., 1994. Major QTLs for post-flowering drought resistance in grain sorghum. *International Plant Genome III Conference*, 1995.

**APPENDIX**

- Table A1. RFLP scores for the 105 elite sorghum inbred lines
- Table A2. RFLP scores of CK60 x PI229828 F<sub>2</sub> plants for all marker loci. Loci are listed in linkage group order.
- Table A3. Plot means and adjusted entry means for morphological traits.
- Table A4. Analysis of variance for morphological traits.
- Table A5. Precipitation amounts and monthly accumulations site averages and deviations from the site average in 1992 and 1994.
- Figure A1. RFLP linkage map of sorghum F<sub>2</sub> population CK60 versus PI229828 used for QTL analyses.

Table A1. RFLP scores for each of the 105 sorghum elite inbred lines.

[illegible]

Table A1. (continued)

[illegible]

Table A1. (continued)

[illegible]

Table A1. (continued)

PROBE	BD	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
BNL08.35E	5	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	1	0	1	1	0	1	0	0	0	1	0	0	1	1	0	1	1	1	0	1	1	1	0	0	0	0	
BNL08.35E	6	1	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
BNL08.35E	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
BNL09.08H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BNL09.11E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BNL12.06E	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BNL12.06E	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BNL12.06E	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BNL14.07H	1	0	0	0	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BNL14.07H	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BNL15.21E	1	1	0	0	0	1	1	1	1	0	0	0	1	0	1	0	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	0	0	0	1	0
BNL15.21E	2	1	1	1	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BNL15.21E	3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BNL15.21E	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EL142H	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EL142H	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EL142H	3	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EL142H	4	1	1	0	0	1	0	0	1	1	0	1	1	0	1	1	1	1	0	0	1	1	0	1	1	1	1	0	0	1	1	1	0	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	0	1
EL173H	1	-	0	-	0	1	0	-	1	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	1	1	1	1	
EL173H	2	-	1	-	1	0	1	-	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EL173H	3	-	1	-	1	0	1	-	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JC0106E	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JC0106E	2	1	1	1	1	1	1	0	1	1	0	1	1	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JC0106E	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JC0106E	4	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JC0106E	5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JC0106E	6	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JC0106E	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JC0178E	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JC0178E	2	1	1	0	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JC0182E	1	0	1	0	0	1	1	0	0	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JC0182E	2	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JC0182E	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JC0182E	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JC0185E	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
JC0185E	2	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table A1. (continued)

[illegible]



Table A1. (continued)

[illegible]

Table A1. (continued)

Table A1. (continued)

[illegible]

Table A1. (continued)

[illegible]

Table A1. (continued)

[illegible]

Table A1. (continued)

[illegible]

Table A1. (continued)

PROBE	BD	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105			
JC0716H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
JC0716H	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
JC0734E	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
JC0734E	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
JC0734E	3	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
JC0775H	1	0	0	1	0	0	1	1	0	0	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	0	0	0	1	0	1	1	0	1	1	1	1	1	1	0	
JC0775H	2	1	1	0	1	1	0	0	1	1	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	
JC0775H	3	1	1	0	1	1	0	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	
JC0783E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
JC0783E	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
JC0813H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
JC0813H	2	0	1	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
JC0813H	3	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
JC0813H	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
JC0813H	5	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
JC0813H	6	0	1	0	0	0	1	0	0	1	1	1	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
JC0827H	1	1	1	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0	1	1	0	1	1	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1	0	0	0	0	0	0	
JC0827H	2	1	0	0	1	0	1	1	1	1	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
JC0859H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
JC0859H	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
JC0891E	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
JC0891E	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0</																												

Table A1. (continued)

[illegible]



Table A1. (continued)

PROBE	BD	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
JC0991H	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
JC0991H	2	0	0	0	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
JC0991H	3	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A1. (continued)

[illegible]

Table A1. (continued)

PROBE	BD	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105		
NPI327H	2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
NPI327H	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
NPI379H	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
NPI379H	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI10-5E	1	0	0	0	0	0	1	1	1	0	0	1	1	0	1	1	1	1	1	0	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
PHI10-5E	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI10-5E	3	1	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI15-37H	1	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI15-37H	2	1	1	-	1	1	0	1	0	1	0	1	1	0	1	1	0	1	0	1	1	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1		
PHI15-37H	3	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI15-37H	4	1	0	-	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-566E	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-566E	2	1	1	0	0	1	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1		
PHI20-589E	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-589E	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-589E	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-589E	4	1	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
PHI20-608H	1	0	0	-	1	0	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1		
PHI20-608H	2	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-608H	3	0	1	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-608H	4	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-608H	5	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-608H	6	1	0	-	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-726H	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-726H	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-726H	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-726H	4	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
PHI20-726H	5	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-728E	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-728E	2	1	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-728E	3	0	1	0	1	1	1	0	1	0	1	0	1	0	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
PHI20-728E	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PHI20-728E	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
UMC005E	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
UMC005E	2	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
UMC005E	3	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

Table A1. (continued)

Table A1. (continued)

[illegible]

Table A1. (continued)

[illegible]

Table A1. (continued)

PROBE	BD	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	
UMC108E	3	0	0	0	59	0	0	0	63	0	65	66	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	
UMC109E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
UMC113E	1	0	0	0	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
UMC113E	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC113E	3	0	0	0	0	0	0	0	1	1	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC113E	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC113E	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC113E	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC113E	7	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC114E	1	1	0	0	0	1	0	0	0	0	0	66	0	0	69	0	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	0	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
UMC114E	2	1	0	1	1	0	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	
UMC114E	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC114E	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC114E	5	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC119E	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC119E	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC119E	3	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
UMC119E	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC122E	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC122E	2	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	
UMC122E	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC134H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
UMC136H	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC136H	2	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
UMC136H	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
UMC168H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	

BD stands for bands

Table A2. RFLP scores of CK60 X PI229828 F2 plants for all marker loci. Loci are listed in linkage group order.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51		
ISU139	B	A	H	H	B	B	H	A	A	H	H	H	H	H	B	H	H	H	B	B	B	B	A	B	H	B	H	B	B	B	B	H	H	H	H	A	H	A	A	H	H	H	H	B	B	A	H	H	A	H	H		
ISU105	B	A	H	H	H	B	H	A	A	B	H	H	A	H	B	H	H	H	B	B	D	B	B	A	B	H	B	H	H	B	B	H	H	H	H	A	H	A	A	H	H	H	H	B	B	A	H	H	D	H	H	H	
ISU038	A	A	H	H	H	A	A	H	A	B	B	C	A	D	B	H	H	H	C	B	H	H	B	H	H	B	H	H	H	B	H	H	H	A	H	H	H	A	H	H	H	H	B	B	A	B	H	H	H	H	H		
ISU154	A	H	B	H	H	A	A	H	A	B	B	B	A	A	B	H	H	B	H	B	H	H	B	H	H	B	H	H	B	H	H	B	H	H	H	A	H	H	H	H	B	B	H	A	H	H	B	B	H	-	H	H	
ISU095	A	B	B	B	H	A	A	H	A	H	B	B	B	A	H	B	H	B	H	B	H	H	B	H	B	H	B	H	H	B	H	H	B	H	H	A	H	H	H	H	B	B	H	A	H	H	B	B	H	-	H	H	
UMC033	A	B	B	B	H	A	A	H	A	B	B	B	H	B	B	H	H	B	H	B	B	B	A	H	B	B	H	B	H	H	B	A	H	H	A	H	H	H	H	B	B	H	A	H	B	H	H	H	B	H	H		
ISU123	A	B	B	B	H	A	H	H	A	B	H	B	H	B	H	H	H	H	B	B	B	B	A	H	B	B	H	B	H	H	H	H	H	H	B	A	H	H	H	H	B	B	H	B	A	H	B	H	H	C	H	H	
ISU116	A	B	B	H	H	A	H	H	H	H	H	H	B	B	H	A	H	H	B	B	B	H	A	H	B	B	H	B	H	H	H	H	H	H	B	A	H	H	H	H	B	B	H	H	H	H	B	B	H	H	B	H	H
ISU074	H	H	A	B	H	A	H	H	H	H	H	H	B	H	B	H	H	H	B	B	H	A	H	B	B	B	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H	B	H	B	H	B	-	H	H		
ISU052	H	H	A	B	H	A	H	H	H	H	H	H	B	A	B	H	H	H	B	B	H	A	A	H	H	B	B	B	H	H	H	H	H	H	H	B	H	H	H	A	H	B	H	A	B	H	H	B	C	H	H		
PIO20568	H	H	H	B	H	B	B	H	H	B	H	B	H	B	H	H	H	A	H	H	H	B	H	B	H	B	B	H	H	H	A	H	B	B	H	H	H	B	B	B	B	H	H	H	H	H	H	B	B	H	H	H	
ISU147	H	H	B	H	B	B	H	H	B	B	H	B	H	B	H	H	A	A	H	B	H	H	B	H	B	H	B	H	H	H	B	H	H	B	H	H	B	B	B	B	H	H	H	H	H	H	B	B	H	H			
ISU155	H	H	B	B	B	A	H	A	H	H	B	H	B	A	B	H	H	A	A	H	B	H	B	A	B	H	H	H	H	H	H	H	H	B	H	H	B	H	H	B	B	H	B	B	A	A	A	A	A	H	H		
UMC071	H	H	B	B	B	A	A	H	H	B	A	H	A	B	H	H	B	H	C	H	B	A	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	B	A	B	B	B	B	A	D	A	-	H	H	-	H	H
ISU058	H	H	B	B	B	A	A	A	H	H	H	H	A	B	H	H	B	H	H	B	H	B	A	H	H	H	H	B	A	H	H	H	H	H	H	H	B	H	A	H	B	B	B	B	A	A	H	A	A	H	H		
ISU030	H	H	B	B	B	A	A	H	H	H	H	A	B	H	H	B	H	B	H	B	H	B	A	H	H	H	B	H	H	H	H	H	H	H	H	H	H	B	H	A	H	B	B	B	A	A	H	A	A	H	H		
ISU138	H	H	B	B	H	A	A	H	H	H	H	A	B	H	B	H	B	H	B	H	B	A	B	H	H	B	B	H	H	B	H	H	H	H	H	H	H	B	A	H	B	B	A	H	A	H	A	H	H	-	H	H	
ISU126B	A	A	A	C	C	C	A	C	A	C	A	C	A	C	C	C	C	C	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C		
ISU135	D	D	D	B	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D		
ISU075	D	A	B	H	H	A	B	H	H	A	H	A	A	H	H	H	B	A	H	H	H	B	A	B	H	H	H	B	A	A	B	A	A	B	A	B	A	H	H	B	A	B	H	B	H	A	H	A	H	H	-	H	H
NPI327	H	A	A	H	H	H	A	C	D	D	A	B	A	A	-	-	B	A	A	H	H	B	A	B	H	B	B	H	B	A	A	B	H	H	H	H	C	A	B	B	B	H	H	H	H	A	-	B	H	-	B	H	
ISU083	H	A	A	H	H	B	A	B	H	B	A	B	A	H	H	H	H	A	A	H	H	B	A	H	B	B	H	B	B	H	B	B	A	B	B	H	H	B	B	B	H	H	H	H	A	A	H	H	-	H	H		
ISU027	H	H	B	B	H	H	H	B	B	H	A	H	A	H	A	H	A	H	H	B	B	B	A	A	H	A	A	B	B	B	H	B	H	H	H	H	B	B	H	B	B	H	H	A	A	C	H	H	-	H	H		
NPI209	A	A	B	B	A	H	H	B	A	B	H	H	H	A	B	H	H	H	B	B	B	A	A	H	B	A	A	B	H	A	B	H	A	B	H	B	B	H	B	B	H	H	H	H	A	H	H	C	H	H	-	H	H
BNL05.09	A	A	B	B	A	H	H	B	A	B	H	H	H	A	B	H	H	H	B	B	B	A	A	H	B	H	H	H	B	A	B	H	H	H	H	B	A	B	H	B	H	H	H	H	A	H	H	H	B	H	-	H	H
ISU040	A	A	B	B	A	H	H	H	H	H	H	H	A	B	H	H	H	A	B	B	H	A	A	H	B	H	H	H	B	H	B	H	B	H	H	H	H	B	B	H	H	H	A	A	H	H	-	H	H	-	H	H	
BNL12.06	A	A	B	H	H	H	H	H	H	H	H	H	B	B	H	A	A	B	B	H	A	A	B	H	B	H	H	B	B	B	H	H	H	H	H	H	B	B	H	H	A	A	H	H	-	H	H	-	H	H			
BNL08.17	A	A	B	H	H	H	H	A	H	H	H	B	B	B	H	A	H	H	A	A	H	H	A	A	B	H	H	H	B	B	H	H	H	H	H	H	H	B	B	H	H	A	A	H	H	-	H	H	-	H	H		
ISU061	A	A	B	A	H	H	H	B	A	H	H	H	A	B	B	H	H	A	A	H	H	H	A	A	H	H	H	H	B	B	B	H	B	H	H	H	H	H	B	B	H	H	H	A	A	B	H	H	-	H	H		
ISU078B	A	A	B	A	H	B	H	H	B	A	H	A	A	B	B	H	A	D	-	H	H	A	A	H	H	H	H	B	B	B	B	H	H	H	H	H	B	B	H	H	H	A	A	B	B	H	A	A	H	H	-	H	H
ISU098	A	A	B	A	H	B	H	A	B	A	H	A	A	B	B	H	H	A	A	H	H	A	A	H	H	H	H	B	B	B	H	B	H	H	H	H	H	H	B	B	H	H	A	A	B	B	H	D	H	H	-	H	H



Table A2. (continued)

	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102		
ISU139	D	B	H	H	B	H	A	B	H	H	B	B	H	H	H	B	A	H	H	A	A	A	A	A	H	H	H	B	H	B	H	H	A	H	B	H	H	B	B	A	H	B	H	H	B	H	B	H	B				
ISU105	H	B	H	H	B	H	A	B	H	H	B	B	H	H	H	B	A	H	H	A	H	H	A	D	H	A	H	B	H	B	H	B	H	H	A	A	B	H	H	B	B	H	A	H	B	B	B	H	H	H	B		
ISU038	H	B	H	H	B	H	A	H	H	H	B	B	H	H	B	A	H	H	A	H	H	A	H	H	H	B	A	H	B	B	H	A	A	B	H	B	B	B	H	A	B	B	B	B	A	B	A	H	H	H	B		
ISU154	H	H	H	H	B	H	-	B	C	A	B	B	H	H	-	B	A	H	H	A	H	H	H	-	H	H	H	B	A	H	B	H	H	A	B	H	B	B	B	H	H	B	B	B	B	A	B	A	H	H	B		
ISU095	H	H	H	B	B	H	A	H	H	A	H	B	H	H	H	H	A	H	A	H	H	H	-	H	H	H	H	B	H	H	A	B	H	B	B	B	B	B	H	B	B	B	B	A	B	A	H	H	H	B			
UMC033	H	H	H	C	B	-	A	H	H	A	H	B	H	H	H	B	A	H	H	A	H	H	C	B	H	B	H	H	B	H	H	B	H	H	B	B	B	B	H	B	H	B	B	A	B	A	H	H	H	B			
ISU123	A	H	H	B	B	H	A	H	H	A	A	B	B	A	H	B	H	H	H	A	H	H	H	B	B	H	B	H	A	B	H	H	B	H	B	B	B	B	H	B	A	B	H	B	B	A	H	H	B	B	H		
ISU116	H	A	A	H	B	B	A	B	H	H	A	B	H	H	B	H	H	H	H	H	H	H	H	B	B	B	H	A	H	H	B	B	H	B	B	B	B	B	B	B	A	H	H	B	B	H	H	H	B	B	H		
ISU074	H	A	H	B	B	H	B	B	H	H	H	-	H	B	H	H	H	A	H	A	-	H	B	H	H	A	H	H	A	B	A	B	B	B	B	B	B	B	B	B	H	H	B	B	H	H	H	H	B	B	H		
ISU052	H	H	A	C	C	C	H	B	H	H	A	H	H	-	B	H	H	A	B	A	H	A	B	H	B	H	H	A	H	H	A	B	B	A	B	B	B	B	B	B	H	H	H	B	H	H	H	B	B	H			
PIO20568	A	B	B	H	B	B	B	H	H	H	B	B	B	B	H	H	A	H	H	B	H	H	B	B	B	B	B	H	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H		
ISU147	B	B	B	H	B	B	B	H	H	B	H	H	H	A	H	A	B	H	B	B	H	A	H	B	B	B	B	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H		
ISU155	B	H	B	H	B	B	B	H	H	H	H	H	H	H	B	H	A	B	H	B	H	H	H	H	H	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H		
UMC071	B	H	H	B	B	B	B	H	H	H	H	A	H	A	B	H	A	H	H	B	A	A	-	H	H	A	H	H	B	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H		
ISU058	B	H	A	H	B	B	B	A	H	H	H	A	H	A	B	H	A	H	B	A	A	H	H	B	A	H	H	A	A	H	H	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H		
ISU030	B	H	A	H	B	B	B	A	H	H	H	A	H	A	B	H	A	H	B	A	A	-	H	A	A	H	H	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	
ISU138	B	H	A	H	B	A	B	A	H	H	H	A	H	H	H	A	H	B	H	A	B	H	A	H	H	B	A	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	
ISU126B	A	C	-	C	C	A	C	C	C	C	C	C	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	C	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C		
ISU135	D	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	B	D	B	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
ISU075	H	H	A	H	A	H	A	B	H	B	A	H	A	H	A	B	H	A	B	H	H	H	H	H	B	A	H	A	B	B	A	A	H	B	H	B	H	B	H	A	H	B	B	H	B	H	B	A	H	H	H	B	
NPI327	H	H	H	H	D	A	A	H	B	H	-	D	A	-	H	A	-	-	A	A	H	B	-	A	H	A	B	D	A	B	H	H	B	H	B	B	B	B	A	H	B	B	B	B	B	B	B	A	H	H	H	H	
ISU083	H	H	B	A	A	A	B	B	H	H	A	H	A	B	H	A	B	A	H	A	B	H	A	B	H	H	A	H	H	B	B	B	B	B	B	B	B	B	B	A	H	B	B	B	B	B	B	B	B	B	B		
ISU027	H	H	H	B	B	B	H	B	H	H	H	B	A	H	A	H	A	B	H	H	H	H	H	H	H	B	A	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	
NPI209	H	H	B	H	B	B	H	B	A	H	H	B	A	H	A	A	A	H	H	B	A	H	A	H	B	A	A	H	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	
BNL05.09	H	H	B	H	B	B	H	B	A	H	H	B	A	H	A	A	A	H	H	B	A	H	B	H	H	B	A	H	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	
ISU040	H	H	B	H	B	A	H	B	A	A	H	B	A	H	A	A	H	H	B	A	H	D	A	H	H	A	A	H	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	
BNL12.06	H	H	B	B	B	A	H	A	A	C	H	B	-	H	A	A	H	H	B	B	A	H	-	A	H	A	A	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	
BNL08.17	H	A	B	B	B	A	H	A	H	H	B	A	H	A	H	H	H	A	B	A	H	A	A	A	H	H	H	A	B	H	A	A	H	H	A	H	H	A	H	H	A	H	H	B	B	B	B	B	B	B	B		
ISU061	H	A	B	B	B	C	A	H	A	H	B	B	-	H	H	A	H	H	A	B	A	-	A	H	A	A	H	H	H	A	B	H	A	A	H	H	A	H	H	A	H	H	A	H	H	B	B	B	B	B	B	B	
ISU078B	H	A	B	H	B	H	A	H	A	H	H	B	A	H	B	A	H	H	H	A	B	A	A	A	A	H	H	H	H	A	B	H	A	A	H	H	A	H	H	A	H	H	A	H	H	B	B	B	B	B	B	B	
ISU098	H	A	B	H	B	H	A	H	A	H	B	A	H	B	A	H	H	A	A	B	D	A	A	H	A	H	H	A	B	H	H	A	B	H	H	A	H	H	A	H	H	A	H	H	B	B	B	B	B	B	B	B	B

Table A2. (continued)

	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	
ISU139	H	H	H	A	A	A	A	H	H	B	H	H	B	H	A	B	H	A	H	H	A	B	B	-	-	H	H	B	H	H	-	B	A	A	H	A	H	B	A	A	B	H	B	A	H	H	H	H			
ISU105	H	H	H	A	A	B	A	H	H	B	A	H	B	H	A	B	H	A	B	H	A	B	B	H	H	H	H	B	H	H	H	B	A	A	H	A	H	B	A	H	B	B	B	A	H	B	H	H	A		
ISU038	H	H	H	H	H	B	H	B	A	B	A	H	H	H	H	B	H	A	H	H	H	H	B	B	H	H	B	H	B	H	A	H	B	A	A	H	H	A	H	B	A	B	B	B	A	A	B	H	A	H	
ISU154	H	H	H	H	H	B	H	B	A	B	A	H	H	H	H	B	H	A	H	H	H	H	B	B	B	H	B	H	A	H	B	A	A	H	H	A	H	B	H	B	B	B	H	H	A	B	H	A	H		
ISU095	H	H	H	H	H	B	H	B	A	B	A	H	H	A	H	B	B	B	H	H	H	H	H	B	B	B	H	H	A	A	H	H	A	H	H	A	H	B	H	B	H	B	H	H	A	B	H	A	H		
UMC033	H	B	H	H	H	B	H	B	A	B	A	H	B	A	H	B	B	H	H	H	H	H	B	H	B	H	H	A	A	H	H	A	H	H	A	H	H	A	H	B	H	B	B	H	H	A	B	H	A	H	
ISU123	H	B	A	H	H	B	H	B	A	B	H	B	A	H	B	B	H	A	A	B	H	H	B	H	B	H	B	H	A	H	A	H	H	H	H	A	H	B	H	B	H	B	H	H	A	H	A	H	A		
ISU116	H	B	A	H	H	B	H	B	H	H	H	A	A	B	H	H	A	A	B	H	H	A	B	H	H	A	H	H	A	H	H	A	H	B	H	H	B	A	B	H	H	B	H	B	H	H	H	A	H	A	B
ISU074	H	H	A	H	B	B	H	B	H	H	H	H	A	B	H	H	A	H	B	B	B	A	H	H	A	H	H	A	H	H	A	B	B	H	A	H	H	B	H	B	H	H	A	B	H	A	H	A	B		
ISU052	H	H	A	H	B	B	H	B	H	B	H	H	H	B	A	B	H	H	A	H	H	B	A	H	H	A	H	H	A	H	H	A	H	B	H	A	H	H	B	H	B	H	H	A	B	H	A	H	-	B	
PIO20568	B	B	B	H	H	B	H	B	H	B	B	B	B	B	H	B	B	B	H	H	H	B	B	H	B	H	B	H	B	B	H	B	B	H	H	H	B	B	H	B	H	H	A	H	H	B	B	H			
ISU147	B	B	B	H	H	B	H	H	B	B	A	B	B	B	H	B	H	H	H	H	H	B	B	H	B	H	H	H	H	H	H	B	B	B	H	H	B	H	H	H	H	H	H	H	H	B	B	B	H		
ISU155	H	B	B	B	A	H	H	H	B	A	H	H	B	H	A	H	A	H	A	H	H	B	B	H	B	H	H	B	B	B	H	B	H	B	H	H	H	H	B	H	H	H	H	H	H	H	H	H	H	H	
UMC071	H	B	B	B	A	H	H	H	B	A	H	H	B	H	A	H	H	H	H	H	B	H	B	B	H	B	H	B	H	B	H	B	H	B	H	H	H	B	H	H	H	H	H	H	H	H	H	H	A	H	
ISU058	H	B	H	B	A	A	H	H	H	B	A	B	H	B	H	H	A	H	H	H	A	H	B	B	B	H	B	H	B	H	B	H	B	H	H	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	
ISU030	A	B	H	B	A	A	H	H	H	B	A	B	H	B	H	A	A	H	H	A	H	B	H	B	B	H	H	H	H	H	B	H	H	B	H	H	H	B	H	H	B	H	H	H	H	H	H	H	H	A	H
ISU138	A	B	H	H	A	A	B	H	B	A	B	H	B	H	H	A	A	H	B	A	H	B	H	B	H	H	H	H	A	H	H	H	A	H	H	H	B	A	H	B	A	H	H	H	H	H	H	H	H	D	H
ISU126B	A	C	C	A	A	C	A	C	C	C	C	C	C	C	C	C	C	C	A	A	C	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	C	C	A	C	C	C	C	C	C	A	C	
ISU135	D	B	D	D	D	D	B	D	B	D	B	D	D	B	D	D	D	D	D	D	D	B	-	-	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
ISU075	A	B	H	A	A	H	H	H	B	A	H	B	B	H	H	H	A	B	H	B	H	H	H	A	H	H	H	A	H	H	H	H	H	H	H	H	H	B	A	H	A	H	B	D	H	H	B	H	D	H	
NPI327	H	B	B	H	H	B	A	H	H	H	A	B	H	H	B	H	A	H	H	A	B	H	B	-	-	B	H	A	H	H	H	A	B	H	H	B	A	H	H	H	A	H	H	H	B	H	-	-	-	H	
ISU083	H	H	B	B	B	H	H	A	H	A	H	H	B	H	A	H	H	H	B	H	B	H	B	H	B	H	H	A	H	H	H	A	B	H	A	B	H	H	H	A	H	H	H	B	A	H	H	B	A	H	
ISU027	H	H	H	H	H	H	A	A	H	A	H	H	B	B	B	A	H	H	B	B	A	H	H	B	H	H	A	A	H	H	H	B	H	A	A	H	A	A	B	A	H	B	A	H	H	H	H	H	H		
NPI209	B	B	B	H	B	H	B	A	H	H	H	A	H	B	B	B	H	A	H	H	B	A	A	B	H	H	B	B	B	H	H	H	B	B	H	H	H	H	A	H	B	B	B	H	H	H	H	H	H	B	
BNL05.09	B	B	H	H	B	H	B	A	H	H	H	A	A	B	B	B	H	A	H	H	B	A	A	B	H	H	H	B	B	B	H	H	B	H	H	H	H	H	A	B	B	B	H	H	H	H	H	B	H	B	
ISU040	B	B	H	B	H	H	B	A	A	H	H	H	A	B	B	B	H	A	H	H	B	A	A	B	H	B	H	H	B	H	H	H	B	H	H	H	H	H	H	H	B	B	B	B	H	H	B	H	B		
BNL12.06	B	B	H	B	H	A	A	H	H	H	B	H	A	B	B	B	H	A	A	H	A	A	B	H	B	H	H	B	H	H	B	H	H	H	B	H	H	A	C	C	C	C	H	H	H	B	H	B			
BNL08.17	B	B	H	B	H	A	A	H	A	H	B	A	B	B	H	A	B	B	A	H	A	A	H	B	H	B	H	B	H	B	H	B	B	A	B	H	H	A	B	H	B	B	H	H	A	H	A	H	B		
ISU061	B	B	H	B	B	H	H	A	H	H	B	B	H	A	B	B	H	A	H	B	A	H	A	H	H	B	H	B	H	B	H	B	A	B	H	H	H	H	B	H	B	B	H	H	A	H	C	B	B		
ISU078B	B	B	H	B	B	B	H	A	B	H	B	B	H	A	B	B	H	A	B	A	H	A	H	B	H	B	H	H	B	B	H	A	B	H	A	H	H	B	B	B	B	H	B	B	H	H	A	H	B	B	
ISU098	B	B	H	B	B	H	A	B	H	B	B	H	A	B	B	H	A	B	B	A	H	A	H	B	B	H	H	H	B	B	A	B	H	A	H	H	H	B	B	B	H	B	B	H	H	A	H	A	B	B	

Table A2. (continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51		
NPI43B	B	A	B	A	B	B	H	A	B	A	H	A	H	H	B	C	H	B	H	H	H	H	H	A	A	H	H	H	H	C	H	A	B	H	H	H	B	H	H	H	A	A	B	B	H	-	A	H					
ISU073B	B	A	B	A	B	B	H	A	B	H	A	A	H	H	H	B	H	B	H	H	H	H	H	A	A	H	H	H	H	B	H	A	B	H	H	H	B	B	H	H	H	A	A	B	B	H	H	H	H				
ISU073A	B	D	B	H	B	B	H	A	B	H	H	H	H	H	H	B	B	H	B	D	H	H	H	H	D	H	H	H	H	H	B	D	B	D	H	H	B	B	H	H	H	H	A	B	B	H	C	H	H				
ISU122	B	H	A	A	B	B	H	A	B	H	A	H	H	H	H	B	H	B	H	H	H	H	H	H	A	A	H	A	H	H	H	B	H	A	B	H	B	H	B	B	H	H	H	A	A	B	B	H	H	H			
ISU119	B	H	A	A	B	D	A	A	B	D	B	A	H	-	A	B	D	B	H	H	-	H	H	A	H	A	H	A	H	H	A	B	H	H	H	B	H	B	H	H	H	H	A	A	H	B	-	H	H				
ISU078A	H	H	A	A	B	H	A	A	B	H	B	A	H	H	A	H	H	B	A	B	A	H	H	A	H	H	A	H	H	A	B	B	H	H	H	B	B	H	H	H	H	H	A	A	H	B	A	-	B	H			
ISU104	A	H	A	H	B	H	A	H	B	B	B	A	H	H	A	H	H	B	A	B	A	H	H	B	B	H	A	H	B	A	B	B	B	H	H	B	B	H	H	H	B	A	H	H	H	H	A	A	B	B	H		
ISU141	A	H	A	H	B	B	A	H	B	B	B	A	H	H	A	H	H	H	A	B	A	H	B	B	H	B	B	A	H	B	A	B	B	B	H	H	B	B	H	B	A	H	H	B	H	A	A	B	B	H			
ISU092	A	B	H	H	H	B	A	B	B	B	B	H	B	H	A	B	B	B	H	B	H	B	B	H	B	B	H	A	B	A	B	H	B	B	H	H	H	H	H	B	B	H	H	H	B	A	A	H	B	B	H		
ISU070	A	B	H	H	B	B	A	B	B	H	H	H	B	H	A	B	B	H	B	A	B	B	B	A	B	B	A	B	H	H	H	H	B	A	H	H	H	H	B	H	H	H	B	H	H	B	A	B	H	B	H		
ISU062	A	B	H	H	B	A	B	B	H	H	H	B	B	A	B	B	B	H	B	A	B	B	H	A	H	B	H	H	H	H	B	A	B	H	H	H	H	H	B	B	H	H	B	B	A	A	B	-	B	H			
ISU067	A	C	H	H	H	C	A	B	B	H	H	H	H	A	C	C	H	H	B	A	C	C	H	A	H	H	H	H	H	C	A	C	H	H	H	H	H	H	C	H	H	H	C	C	A	A	C	-	C	H			
ISU023	B	A	B	A	H	B	A	B	B	B	A	H	H	H	B	H	B	A	H	H	A	H	H	B	B	H	B	H	H	B	H	A	H	H	A	H	H	B	A	A	B	H	B	A	B	H	H	H	H	H			
BNL05.40	B	A	B	A	H	B	A	H	H	B	A	H	H	B	B	B	A	H	H	A	H	H	B	H	H	B	H	H	H	H	A	H	H	H	H	B	A	B	H	H	A	A	B	B	H	A	H	A	H				
PIO20566	B	A	B	A	H	H	A	H	A	B	H	H	H	B	B	B	A	H	A	H	H	H	H	H	H	H	B	H	B	H	A	H	H	B	H	H	B	B	A	B	A	A	H	A	A	B	B	H	A	H			
ISU126A	B	A	B	H	H	H	A	H	B	H	H	H	H	B	B	B	H	H	H	H	H	H	H	H	H	H	B	H	H	H	A	H	B	H	H	B	B	H	B	B	H	H	A	A	H	B	B	H	H				
ISU042	H	A	H	A	A	H	H	A	B	H	B	H	A	B	B	B	H	H	H	H	B	H	H	B	H	H	B	B	A	H	B	H	H	B	B	H	B	A	B	A	A	H	A	A	B	B	H	A	H				
NPI104A	H	A	H	H	A	H	B	H	A	H	B	B	H	H	B	B	H	B	H	B	B	B	H	A	H	A	H	A	B	H	H	A	A	B	A	H	H	B	B	A	A	H	H	H	B	H	C	H	H				
ISU080	H	H	H	H	H	B	H	A	H	B	B	A	H	B	B	B	B	B	H	C	B	H	A	H	A	A	B	H	H	A	A	B	H	H	H	B	B	A	B	A	H	H	H	H	B	B	H	H	H				
ISU056	B	H	B	H	A	H	B	A	H	B	H	H	B	B	B	B	B	B	H	B	B	H	B	B	H	H	A	H	B	H	H	H	B	B	B	B	B	H	B	B	A	B	A	H	H	B	A	H	B	A	H		
PIO20608	B	H	B	H	A	B	B	A	D	B	H	B	H	A	H	H	B	B	B	H	H	H	B	H	H	H	H	H	H	H	H	B	B	B	H	A	B	H	A	A	A	B	B	H	H	H	H	H	H				
ISU107	B	H	B	H	B	B	A	H	H	H	B	H	A	H	B	H	B	H	H	B	H	H	B	H	B	H	B	H	H	A	B	B	B	B	B	B	B	H	H	A	A	A	B	H	H	H	H	H	H				
ISU140	B	A	H	A	A	B	H	H	B	H	H	B	H	A	A	B	A	B	B	H	A	A	H	A	A	H	A	H	H	B	A	H	H	B	A	H	H	B	B	A	H	H	B	B	H	H	B	B	H	H			
PIO1016	B	A	H	A	A	H	H	B	B	H	B	H	A	A	H	H	B	A	B	B	B	A	H	A	A	H	A	H	A	B	A	H	H	B	H	B	H	B	B	H	H	H	H	B	H	A	B	B	H	H			
PIO20714	H	A	H	A	A	H	H	B	A	H	B	H	A	H	B	B	A	H	B	H	A	A	B	B	A	A	B	A	B	H	A	A	B	A	B	A	H	H	B	B	H	H	H	H	H	B	H	A	B	H	-	H	H
NPI560	H	A	H	A	A	H	H	B	A	H	B	H	A	H	B	H	B	A	H	B	A	H	H	A	A	B	A	B	A	A	H	H	B	B	H	B	B	H	H	H	H	H	H	B	A	A	B	B	H	H	H		
ISU026	H	A	H	A	A	H	H	B	A	H	H	A	H	B	H	B	A	B	B	H	A	A	B	B	A	A	B	A	H	B	A	A	H	B	A	H	H	H	H	H	B	B	A	H	B	H	H	H	H	H			
UMC064	A	A	H	A	A	H	H	A	H	A	H	H	A	A	A	B	B	B	H	B	A	B	B	H	B	A	B	A	H	H	B	A	H	B	A	H	H	A	B	H	B	H	H	H	H	B	B	H	H	H			
ISU032A	D	A	H	A	H	H	H	B	A	A	H	H	H	A	A	B	B	B	H	B	A	B	B	B	H	H	A	B	A	A	H	A	H	H	B	B	H	H	H	H	H	H	H	H	H	H	B	H	A				
ISU072	A	A	A	A	H	H	H	B	A	A	H	H	B	H	A	A	B	B	B	H	A	B	B	B	H	A	B	B	B	H	A	B	A	H	A	H	H	B	B	B	H	H	A	A	H	H	B	H	H	H			
ISU115	D	D	D	D	B	D	D	B	B	D	D	D	B	B	D	D	D	B	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D				
BNL06.20	A	H	H	H	B	H	H	H	B	B	A	H	A	H	B	B	B	H	H	H	H	H	H	A	H	H	B	B	A	H	H	H	B	A	H	A	B	H	H	H	H	H	H	H	B	A	A	A	B	H			

Table A2. (continued)

	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102		
NPI438	A	A	B	H	B	H	A	H	H	H	A	H	B	-	B	C	H	D	A	H	A	A	B	-	A	H	H	A	H	A	A	H	H	A	B	H	H	A	H	B	A	H	H	H	B	H	A	H	B	H	A		
ISU073B	A	A	B	H	B	H	A	H	H	H	A	H	B	H	B	B	H	H	A	H	A	A	B	A	A	H	H	A	B	A	H	H	A	A	B	H	H	A	H	B	A	H	H	H	B	H	A	H	B	H	A		
ISU073A	H	H	B	H	B	H	H	H	H	H	H	B	H	B	B	H	A	H	H	A	H	A	B	H	A	H	H	A	B	A	H	H	A	B	H	H	A	H	B	H	H	H	B	H	A	H	B	H	H	H	H		
ISU122	A	A	B	H	B	H	A	H	H	H	A	H	B	B	B	B	H	H	A	H	A	A	B	A	A	H	H	B	A	A	H	A	A	A	B	H	H	A	H	B	H	B	H	A	H	B	H	H	H	H			
ISU119	H	A	B	D	B	D	A	H	H	H	H	H	C	B	B	H	H	H	H	H	A	B	D	A	H	H	D	B	H	A	H	A	A	H	A	H	A	H	B	H	B	H	B	B	D	H	H	B	H	H	H		
ISU078A	H	H	B	B	B	-	A	H	H	H	H	H	H	H	B	B	H	H	H	H	A	B	A	A	H	H	B	B	A	H	H	A	H	A	H	A	H	A	H	B	H	A	B	H	H	H	B	H	H	H	H		
ISU104	H	H	B	B	B	A	A	A	H	H	H	H	H	B	B	H	H	H	B	B	H	H	B	H	H	A	A	H	H	B	B	A	A	B	H	B	A	H	A	B	H	A	B	A	B	H	H	H	H	H	H		
ISU141	H	B	B	B	B	H	A	A	H	H	H	H	H	B	B	H	H	H	B	B	H	H	B	H	H	A	H	H	B	B	A	A	B	H	B	A	H	H	A	B	A	B	A	B	H	H	H	H	H	H	H	H	
ISU092	H	B	B	B	B	H	A	A	H	H	A	B	H	H	H	B	B	H	H	H	B	B	H	H	H	A	H	H	H	H	B	A	B	H	H	H	H	A	H	A	B	H	A	B	H	A	H	H	H	H	H		
ISU070	A	B	B	B	H	H	H	A	H	B	A	H	H	H	H	B	H	H	H	B	B	H	H	H	B	A	B	H	H	H	B	A	B	H	H	H	H	H	A	H	A	H	A	B	H	A	H	H	H	H	H		
ISU062	A	B	H	B	H	H	H	H	B	A	H	H	-	H	D	D	D	A	B	B	H	A	-	C	A	B	H	A	H	B	B	H	H	H	H	H	A	A	A	H	A	B	H	H	H	H	H	H	H	H	H		
ISU067	A	C	H	B	H	H	H	A	H	H	-	H	H	-	H	C	C	H	H	B	C	H	A	-	B	A	H	A	H	C	A	C	H	H	H	H	H	A	H	A	H	A	B	H	H	H	B	H	H	H	H		
ISU023	H	H	B	B	B	B	B	A	H	A	A	H	B	A	H	A	A	H	B	H	H	H	H	A	H	A	B	B	B	H	H	H	H	H	H	H	B	B	B	B	B	A	H	B	H	B	B	A	B	A	B		
BNL05.40	H	H	H	H	B	B	B	A	B	A	A	H	B	A	H	A	A	H	B	H	H	H	H	A	A	B	B	B	B	H	H	H	H	H	H	B	B	B	B	B	B	B	B	B	B	A	B	B	A	B	A		
PIO20566	B	A	H	H	B	B	H	A	B	A	A	H	H	A	B	A	H	B	H	H	H	H	A	H	A	B	H	B	B	H	H	H	H	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	
ISU126A	B	H	H	H	B	B	H	H	B	H	H	H	H	H	B	B	H	H	B	H	H	H	H	H	H	A	B	H	B	B	H	H	H	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	
ISU042	B	H	B	H	B	A	H	H	B	A	A	H	H	A	B	H	H	B	H	H	B	H	A	H	H	B	A	A	B	H	H	B	A	A	H	H	B	B	B	A	B	B	B	A	B	B	A	B	B	H	H	H	
NPI104A	B	B	B	A	H	H	H	H	A	A	H	H	A	H	H	A	H	H	A	H	H	H	H	A	H	H	B	H	A	H	H	H	H	A	A	B	B	B	H	H	H	A	B	H	A	B	H	H	H	A	H	H	
ISU080	B	B	B	A	H	H	-	H	H	A	A	H	H	A	H	H	H	A	H	H	H	H	H	A	H	H	B	H	A	A	H	H	A	A	A	A	B	B	H	H	H	A	B	H	A	B	H	H	A	B	H	H	A
ISU056	C	H	B	H	H	B	A	B	B	A	A	H	B	H	A	H	H	H	B	H	H	H	H	A	H	B	H	B	A	H	A	H	A	A	H	A	A	B	H	A	B	H	A	B	A	B	H	H	H	A	H	A	
PIO20608	C	H	H	H	H	A	H	C	H	A	A	B	D	A	D	D	H	B	H	H	H	D	D	B	B	H	H	H	H	A	H	A	A	H	H	H	H	A	A	B	H	A	H	H	H	H	H	H	H	H	A	H	H
ISU107	B	A	H	H	H	A	H	H	H	A	H	H	A	H	B	H	B	A	H	H	H	B	B	H	H	H	H	H	H	H	A	A	A	H	H	H	A	H	B	H	A	H	A	B	H	B	H	H	H	H	H		
ISU140	B	B	B	H	H	H	B	H	H	B	B	H	H	H	H	B	H	H	H	A	H	H	A	B	H	A	B	B	H	H	H	A	B	H	A	B	H	H	H	A	B	H	H	H	B	B	H	H	H	H	H	H	
PIO1016	B	H	B	H	H	H	B	A	H	B	B	B	H	H	A	H	H	A	A	H	B	H	B	H	B	H	A	B	A	H	A	H	H	A	B	H	H	H	B	B	H	A	H	H	B	H	A	H	H	H	H		
PIO20714	B	H	B	H	H	H	B	A	B	B	B	H	H	A	A	H	H	A	B	B	H	-	B	A	A	H	H	H	H	B	B	H	H	H	B	B	H	H	A	H	H	B	B	A	A	B	H	H	A	B	H	H	
NPI560	B	H	B	H	H	H	B	A	B	B	B	H	H	A	A	H	B	A	B	A	B	B	H	B	B	A	B	A	H	H	B	H	H	B	H	H	C	B	H	H	A	A	H	B	B	A	H	B	A	H	H		
ISU026	H	H	B	H	H	B	B	A	B	B	B	H	H	A	A	H	B	A	B	H	B	B	B	B	A	H	A	H	B	B	H	B	H	B	B	H	H	H	B	H	H	A	A	B	B	H	A	H	B	A	H		
UMC064	H	B	H	H	A	B	B	H	H	B	H	A	A	H	B	A	B	A	H	A	A	B	A	A	H	H	B	B	A	H	B	B	A	H	A	A	A	B	H	B	B	H	B	B	H	A	H	H	A	H	A	H	A
ISU032A	H	B	H	B	B	B	H	A	B	H	A	A	H	H	A	B	D	A	H	B	B	A	A	H	H	B	A	A	H	B	A	H	A	B	H	D	A	A	B	H	B	H	B	H	H	D	H	H	A	H	A	H	A
ISU072	H	B	B	H	B	B	H	H	A	B	H	A	C	H	H	A	B	H	A	H	H	B	B	A	A	H	H	B	A	H	H	B	A	H	A	B	H	A	A	A	B	H	B	B	H	H	H	A	H	H	A	H	A
ISU115	B	D	D	D	D	-	D	D	B	D	D	B	B	-	D	D	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	B	-	D	B	B	D	D		
BNL06.20	B	A	H	H	A	A	H	H	H	A	A	B	B	H	A	H	B	H	B	B	A	H	H	H	H	H	B	H	A	H	B	H	H	B	H	H	H	A	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H

Table A2. (continued)

	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152
NPI438	H	H	H	B	B	H	H	A	A	B	B	H	B	B	H	A	B	H	A	H	H	H	H	H	H	B	H	H	A	H	A	B	H	A	H	H	H	A	H	B	A	B	H	A	H	A	D	-	B	
ISU073B	A	H	H	B	B	H	H	A	A	B	B	H	H	A	B	H	A	B	H	A	H	H	H	H	H	B	H	H	A	H	A	B	H	A	H	H	H	A	H	B	A	B	H	A	B	H	B	B		
ISU073A	H	H	H	B	B	H	H	A	A	B	B	H	H	H	B	H	A	H	H	A	H	H	H	H	H	B	H	H	H	H	H	H	B	H	A	H	H	H	H	B	A	B	H	D	B	H	C	B		
ISU122	A	H	H	B	B	H	B	A	A	B	B	H	A	H	A	H	A	H	H	H	H	H	H	H	H	B	H	H	A	H	H	H	B	H	A	H	H	H	A	H	B	A	B	H	A	B	H	B		
ISU119	H	H	H	B	B	D	B	A	A	H	H	B	A	H	H	B	H	A	H	H	H	H	H	H	H	A	B	H	A	H	H	H	B	H	A	A	H	H	A	H	B	B	H	A	B	A	-	-	B	
ISU078A	H	H	H	B	B	A	B	H	A	A	A	B	H	H	A	H	H	A	A	H	H	H	H	H	A	B	H	H	A	H	H	H	B	H	A	A	H	H	A	H	H	B	B	H	A	D	B	-	B	
ISU104	H	H	H	B	B	H	B	H	H	A	H	B	A	H	H	H	H	H	H	B	A	B	H	H	H	B	H	H	A	B	H	A	H	A	H	A	H	H	H	H	B	B	B	A	H	B	H	B	B	
ISU141	H	B	H	B	B	A	B	H	A	H	B	A	H	H	H	H	H	B	A	B	H	H	B	B	A	B	H	B	H	A	B	H	A	H	A	H	H	H	B	B	B	A	H	H	B	H	C	H		
ISU092	A	B	H	B	A	B	B	H	A	H	H	H	H	B	H	H	A	H	H	B	B	H	B	H	H	B	H	A	B	B	A	A	H	B	H	H	H	B	B	H	B	A	H	H	H	H	H	A		
ISU070	H	B	H	A	B	A	B	A	B	H	H	H	H	B	B	B	A	H	H	B	B	H	B	B	H	H	B	H	A	H	H	A	A	A	H	B	H	H	H	H	B	H	A	H	D	D	D	A		
ISU082	H	B	H	B	H	H	A	B	A	B	B	H	H	A	B	B	B	A	H	B	B	B	B	B	B	A	B	H	A	H	H	H	A	A	H	B	H	H	A	H	B	H	H	A	H	H	H	A		
ISU067	H	H	H	A	C	H	A	H	C	B	H	H	H	A	H	C	B	A	A	B	-	-	-	B	B	A	A	H	H	H	A	A	H	C	H	H	H	H	H	H	H	H	A	H	H	H	A			
ISU023	H	H	A	A	B	H	H	H	B	H	B	H	B	A	A	B	B	A	A	H	H	H	H	B	A	A	B	H	H	H	H	H	B	H	H	A	H	B	A	A	H	B	H	A	B	B	A	A	B	
BNL05.40	A	H	A	A	B	H	H	H	B	H	B	H	B	A	B	B	A	B	B	A	H	H	H	B	B	A	H	A	A	H	H	H	B	H	A	B	A	H	H	H	B	A	B	B	A	B	A	B		
PIO20566	A	H	A	A	B	H	A	H	B	H	B	H	B	A	B	B	A	H	B	A	H	B	A	B	H	A	H	A	A	H	H	H	H	A	H	A	B	A	H	H	B	H	H	B	H	A	B			
ISU126A	A	H	A	A	B	H	A	H	B	H	B	H	B	H	B	H	B	A	H	H	B	B	H	B	H	H	A	H	B	H	H	H	H	H	H	H	B	H	H	H	B	H	B	H	H	A	B			
ISU042	A	H	A	B	H	A	H	A	H	B	H	H	H	H	H	H	A	H	A	H	H	H	H	H	A	H	A	A	B	H	H	H	H	H	A	B	H	B	H	H	B	H	H	H	H	A	H			
NPI104A	A	B	H	B	H	A	H	B	A	H	H	H	H	H	A	H	A	H	A	H	H	H	B	B	A	H	A	A	H	H	H	H	A	A	H	H	B	A	B	H	H	H	H	H	A	H				
ISU080	A	H	B	H	B	H	A	H	B	A	H	H	H	H	B	H	H	A	H	H	B	H	B	A	H	A	H	A	A	H	H	H	H	A	H	H	B	A	A	B	H	H	-	-	-	H	-	-	A	
ISU056	H	H	B	H	A	H	A	H	A	B	H	H	H	H	H	H	H	H	B	H	H	H	H	H	H	H	H	H	A	B	H	H	H	H	H	A	H	H	B	H	A	A	A	B	H	H	H	B	H	
PIO20608	H	H	H	H	H	A	H	A	B	H	H	H	H	H	H	H	H	H	H	B	A	H	H	B	A	H	H	B	A	A	H	B	H	A	A	H	H	B	H	A	A	B	B	H	H	B	H	B		
ISU107	H	A	H	H	H	A	B	H	H	H	H	H	H	H	B	H	B	A	H	A	B	A	B	H	A	B	A	B	H	A	A	A	A	H	H	B	H	A	A	A	B	H	A	B	H	A	B	H		
ISU140	H	B	B	H	B	H	H	H	H	H	A	A	B	H	H	A	A	A	A	H	H	B	H	A	H	A	B	H	B	A	H	B	B	A	H	B	A	H	H	B	H	H	B	B	B	H	B	H		
PIO1016	H	B	B	B	H	H	H	B	H	H	A	A	B	H	H	H	A	A	A	A	A	A	A	A	A	A	H	A	H	A	H	H	H	B	A	H	A	H	B	H	H	H	H	H	B	A	H	B		
PIO20714	A	B	B	H	B	H	B	A	H	B	B	H	B	B	A	A	H	D	-	-	-	-	-	B	A	H	H	A	A	H	H	A	A	A	A	-	B	-	B	-	-	H	A	-	-	-	-	A		
NPI560	A	B	B	H	B	A	B	A	B	B	B	B	A	H	H	H	A	A	A	A	A	A	A	A	H	H	A	H	H	A	H	H	H	A	A	H	B	H	B	H	A	H	A	H	B	A	H	B		
ISU026	A	H	B	B	A	B	A	H	B	H	C	H	A	H	C	H	A	A	A	A	A	A	A	H	H	A	H	H	H	A	H	H	H	A	A	H	A	H	H	H	B	B	H	A	H	A	H	B	B	
UMC064	A	H	B	H	A	B	A	H	H	H	A	B	B	A	H	H	A	A	A	A	A	A	H	A	H	H	H	H	H	H	H	H	A	A	A	H	A	B	A	B	B	A	H	A	H	B	B			
ISU032A	A	B	A	H	B	H	H	B	H	A	H	H	B	H	A	H	A	A	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	B	A	H	B	A	B	A	B	B	A	A	B	B	B	D		
ISU072	H	A	B	H	H	B	H	H	B	H	A	H	H	H	H	A	H	A	A	A	B	H	H	H	H	H	H	H	H	H	H	H	H	B	B	H	B	A	B	B	B	H	A	A	B	B	B	A		
ISU115	B	B	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
BNL06.20	B	H	H	H	B	H	A	A	A	H	A	B	H	B	A	A	A	H	B	B	B	B	A	B	H	B	B	A	B	H	H	B	B	B	B	A	B	A	-	A	-	A	-	H	B	H	B	-		

Table A2. (continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	
BNL14.07	A	A	H	H	H	B	H	H	H	B	A	H	A	H	B	B	B	H	H	H	H	H	H	H	H	H	A	H	H	B	B	B	H	H	H	B	A	B	A	B	H	H	H	H	B	A	A	D	A	B	H	
ISU066	C	A	B	H	H	B	A	H	H	B	B	A	H	A	H	B	B	B	H	H	H	H	H	H	H	H	H	H	B	B	B	B	B	H	H	B	A	B	A	B	B	B	B	H	H	H	H	B	A	A	B	H
ISU037	C	A	B	H	H	B	A	H	H	B	B	A	H	A	H	B	B	B	H	H	H	H	H	H	H	H	H	H	B	B	B	B	B	H	H	B	A	B	A	B	B	B	H	H	H	H	H	A	A	B	H	
ISU117	H	A	B	H	H	B	A	B	H	B	B	A	H	A	H	B	B	B	H	H	H	H	H	H	H	H	H	H	B	B	B	B	B	H	H	B	A	B	A	B	H	B	B	B	H	H	H	A	H	B	H	
ISU066	H	H	B	H	B	A	A	H	H	B	A	A	H	H	H	H	H	B	H	H	H	H	H	H	H	B	B	A	B	H	H	B	B	B	B	B	H	B	B	H	H	B	B	B	B	B	A	B	A	C	H	H
UMC139	B	H	H	H	B	A	A	H	B	B	A	A	A	H	H	H	B	B	H	B	B	H	H	H	B	B	A	B	H	H	B	B	B	B	B	H	H	B	B	H	H	B	B	H	B	H	A	B	H	B	H	
ISU094	B	H	H	H	B	A	A	H	B	H	A	H	A	H	H	H	H	H	B	H	H	H	H	H	B	A	B	A	B	H	H	B	B	B	B	H	B	B	B	B	H	H	B	A	H	H	A	B	H	B	A	H
ISU151	B	B	H	H	B	A	A	B	B	H	A	H	A	H	H	H	H	H	B	H	H	B	B	B	A	B	A	H	H	H	B	B	B	B	H	B	B	B	H	A	H	A	B	A	H	H	A	B	H	B	A	H
ISU071	H	B	H	H	B	A	H	B	H	H	A	H	H	H	A	H	H	H	B	H	H	H	H	B	A	B	A	H	H	H	B	B	H	B	B	H	B	B	B	H	A	H	A	H	A	B	H	B	B	A	H	
ISU130	H	B	H	H	A	H	H	B	H	A	A	H	B	H	H	H	A	B	H	B	D	H	B	A	A	H	H	H	H	B	B	B	B	H	H	A	B	B	C	A	H	H	B	B	A	A	B	A	C	B	H	
NPI400	B	B	H	H	A	H	H	B	H	A	H	H	B	H	H	H	A	H	H	B	H	A	H	A	A	H	H	H	H	B	B	B	B	H	H	B	H	A	B	A	A	A	H	H	B	A	A	B	A	B	B	H
PIO20622	B	H	H	A	H	H	A	H	H	A	A	H	B	B	H	H	H	A	H	H	H	A	H	A	H	H	H	H	B	H	H	B	H	H	B	H	A	H	H	A	H	H	B	A	A	B	A	B	B	H		
ISU021	H	B	A	H	H	H	A	H	B	H	H	B	H	H	A	B	H	B	H	H	B	H	H	A	H	A	A	H	A	A	H	A	H	H	B	B	H	H	B	H	B	B	A	H	A	B	A	H	H	H		
ISU068	B	B	A	H	H	A	H	B	H	H	H	B	B	H	H	H	B	B	B	H	C	B	H	H	A	A	H	A	C	H	H	B	B	B	B	B	H	B	H	B	A	B	B	B	A	B	H	H	H	H		
BNLC6.16	B	B	A	H	H	A	B	B	H	B	B	B	B	H	H	H	B	B	B	B	H	B	B	H	H	H	H	H	H	A	H	B	B	B	B	B	B	H	B	A	B	H	H	B	A	B	H	H	A	C	H	H
NPI212	B	B	A	H	H	H	A	B	B	H	B	B	B	H	H	H	H	B	B	B	H	B	H	H	H	H	H	H	A	H	H	B	B	B	B	H	H	B	B	B	H	B	H	H	H	H	B	B	A	H		
UMC093	B	H	B	A	A	H	A	H	B	H	B	B	B	H	H	B	A	B	H	H	B	H	B	B	B	B	B	B	H	H	A	H	H	B	B	H	H	H	B	B	B	H	B	H	H	A	A	H	B	H	A	
NPI426	B	H	B	A	A	H	A	H	H	B	B	B	B	H	H	H	A	B	H	A	H	B	H	B	B	B	B	B	H	B	H	A	A	H	B	B	A	B	B	B	H	H	H	A	A	H	B	H	A	H		
ISU057	B	D	B	D	D	D	D	D	B	B	B	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	B	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D		
BNL13.05	B	H	A	A	H	A	H	H	B	B	B	B	H	H	A	B	H	A	A	B	B	B	B	H	H	A	A	H	A	A	B	A	H	H	A	B	H	B	B	A	H	H	H	A	H	H	H	A	H	H	H	
BNL09.11	B	H	A	A	H	A	B	B	H	B	B	C	H	A	H	B	H	A	H	B	B	B	B	B	B	B	B	B	B	A	D	H	A	B	A	H	H	H	B	H	B	A	H	H	H	A	H	H	A	B	H	
ISU091	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
BNL03.06	H	H	A	A	H	A	B	H	B	A	H	A	H	H	H	H	B	H	H	A	H	B	H	H	H	B	A	H	A	A	H	H	B	B	A	H	A	A	A	A	A	A	H	B	B	H	A	A	A	H		
ISU079	H	H	A	A	H	A	B	H	B	A	H	A	H	H	H	H	B	H	H	A	H	B	A	H	B	A	H	H	B	A	H	A	A	H	B	B	A	H	H	A	A	A	A	A	H	B	H	H	A	A	H	
ISU136	H	H	A	A	H	A	B	H	B	A	H	A	H	H	H	H	B	H	A	A	H	B	A	H	B	A	H	A	A	H	B	B	B	A	H	H	A	A	H	A	A	A	H	B	B	H	H	A	D	A	H	
PIO10.5	A	H	A	A	H	A	B	H	H	H	A	H	D	A	H	H	B	H	A	D	H	B	A	H	H	B	A	H	A	D	B	B	H	H	H	H	A	A	H	A	A	H	B	H	H	A	D	A	H			
ISU032B	A	H	A	H	A	B	H	H	H	H	A	H	A	H	H	H	H	A	B	H	H	-	H	H	B	A	H	A	A	B	B	H	H	H	H	A	A	-	A	A	B	H	H	H	H	D	H	H				
ISU156	H	H	A	B	A	A	H	H	B	H	A	B	A	H	A	A	A	B	H	A	A	H	B	A	H	H	B	A	H	A	B	B	H	H	H	H	A	H	A	H	A	H	B	H	H	H	A	B	H			
ISU034	A	A	H	A	B	H	A	H	H	H	A	A	B	A	H	H	H	A	A	B	H	A	B	B	A	H	B	A	H	H	A	B	H	H	H	H	A	H	A	A	A	A	H	B	H	H	H	B	H			
UMC114	A	A	H	A	B	H	A	H	H	H	A	A	B	A	H	B	B	H	A	A	B	H	A	B	H	B	A	H	H	A	B	H	H	H	H	H	A	H	A	A	A	B	H	H	A	D	B	H				
ISU110	H	A	H	H	B	H	A	D	A	H	A	A	H	A	H	B	B	B	A	B	B	H	H	A	B	B	B	D	D	D	D	D	B	B	B	A	H	A	D	A	H	A	A	H	H	D	-	B	H			
ISU077	D	D	D	D	B	D	A	D	D	D	D	D	D	D	D	D	B	B	B	D	D	D	D	D	D	B	D	D	D	D	D	D	D	B	B	B	B	D	D	D	D	D	D	D	B	B	D	-	B	D		

Table A2. (continued)

	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102		
BNL14.07	B	A	H	H	A	A	H	H	H	H	A	A	A	B	B	H	A	H	B	H	H	B	A	D	H	H	H	B	B	H	B	H	A	B	H	H	B	B	H	A	H	H	A	A	H	B	B	B	H	A			
ISU066	B	A	A	H	A	-	A	H	B	A	A	H	H	H	B	H	A	A	B	A	B	H	H	A	D	H	H	H	B	B	H	H	A	B	H	B	B	B	H	A	H	H	A	A	H	B	A	B	B	H	A		
ISU037	B	A	A	H	A	B	A	H	B	A	A	B	H	H	B	H	A	A	B	A	B	A	H	H	H	H	H	B	H	H	H	H	A	B	H	H	B	B	H	A	H	H	A	H	B	A	B	B	H	H	A		
ISU117	B	A	A	H	A	B	A	H	B	A	A	B	H	H	B	H	A	A	B	A	B	A	H	A	C	H	H	H	B	H	A	H	A	B	H	H	B	B	H	A	H	H	H	H	B	A	B	B	H	H	A		
ISU086	B	A	A	A	B	A	H	B	A	A	B	H	H	B	A	B	A	B	A	B	A	H	H	A	B	B	B	B	H	A	H	A	B	A	B	B	B	B	A	A	H	B	B	B	A	H	H	B	H	A			
UMC139	B	A	A	H	B	A	H	B	A	H	B	H	H	H	A	B	A	B	A	B	H	H	H	A	B	B	B	H	A	B	H	A	H	A	B	B	B	B	A	H	H	B	B	B	B	A	H	H	B	H	A		
ISU094	H	A	A	H	H	B	H	H	B	A	H	B	H	H	A	A	B	A	B	A	B	H	H	B	B	B	B	H	A	B	H	A	H	H	H	B	B	B	A	H	H	B	H	B	A	A	A	B	H	A			
ISU151	H	A	A	H	B	B	H	H	B	A	H	B	H	H	A	A	B	A	B	B	B	H	B	H	H	H	H	A	H	H	A	H	H	B	H	B	H	B	H	B	H	B	A	A	H	H	H	H	H	H			
ISU071	H	A	A	H	B	B	H	B	B	H	H	B	H	H	A	A	B	A	B	B	B	A	B	H	H	H	H	H	H	H	A	H	B	H	B	H	H	H	A	H	H	H	B	B	B	A	H	H	H	A	H		
ISU130	H	A	H	H	B	A	A	H	B	B	H	H	B	B	A	H	B	H	H	H	A	B	H	H	B	H	H	B	A	B	H	B	B	H	B	B	H	H	A	H	H	B	B	B	H	H	H	H	A	B			
NPI400	H	A	H	H	B	A	A	H	B	B	H	B	B	H	H	B	H	H	H	H	A	B	H	H	H	B	H	A	B	H	B	A	B	H	B	B	A	B	H	H	H	A	H	H	B	B	H	H	B	A	B		
PIO20622	H	A	H	A	B	A	A	H	B	B	H	H	B	B	B	B	H	H	H	A	H	A	H	B	A	H	B	H	A	B	H	B	H	B	H	H	H	A	A	H	H	B	B	H	B	H	B	H	B	A	B		
ISU021	B	H	H	A	H	B	B	H	H	H	A	A	B	A	B	H	A	A	H	B	B	H	A	H	B	H	H	A	B	H	H	A	A	B	A	A	H	H	B	B	H	H	H	H	H	H	H	H	H	H	H		
ISU068	B	H	A	A	H	B	H	H	B	H	A	A	B	A	B	H	H	A	A	H	H	B	H	H	A	A	B	H	A	A	B	H	H	A	A	B	A	H	H	B	B	H	H	H	H	H	H	H	H	H	H		
BNL06.16	H	H	A	A	H	H	A	B	H	B	B	H	B	C	A	B	B	H	A	H	H	H	-	B	A	H	H	A	H	A	B	H	A	B	H	B	B	H	H	H	B	H	H	H	B	H	A	H	H	H			
NPI212	H	H	A	A	B	H	A	B	H	B	B	H	B	B	A	B	B	A	A	H	H	H	H	B	A	H	A	H	A	H	A	B	H	A	H	B	A	H	H	H	H	H	H	H	B	H	H	A	H	H			
UMC093	A	H	H	B	B	H	A	B	H	A	B	A	B	H	H	H	H	A	A	H	B	A	H	H	B	B	A	A	H	A	B	H	B	B	H	H	A	A	H	H	A	H	B	A	H	H	A	H	A	A			
NPI426	A	H	H	H	B	B	H	B	H	A	B	H	H	B	H	H	H	A	H	B	A	H	C	A	B	B	B	B	H	A	H	A	B	B	B	H	H	H	A	A	H	H	A	H	H	A	H	A	H	A			
ISU057	D	D	D	D	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	B	B	B	D	D	D	D	D	B	B	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D		
BNL13.05	H	H	H	H	A	H	H	B	H	A	B	H	H	B	H	H	H	A	H	B	A	B	-	A	H	B	B	H	A	H	B	B	A	H	H	B	B	A	H	H	H	H	B	A	B	A	H	B	A				
BNL09.11	H	H	B	H	A	H	H	B	H	A	B	B	H	B	H	H	H	H	A	H	B	H	B	H	A	A	H	B	H	A	B	H	A	B	H	B	H	A	B	B	H	B	B	A	B	A	B	B	H	B			
ISU091	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	H	H	H	H	B	H	A	A	B	B	H	A	B	A	B	H	B	H	A	H	B	H	A	B	B	H	B	A	B	A	H	B	H			
BNL03.06	H	H	A	B	H	A	H	A	B	B	B	A	A	H	H	B	H	H	H	H	H	B	D	B	B	H	A	H	H	A	H	H	A	H	B	B	H	A	H	B	B	A	B	A	B	B	B	H	H				
ISU079	H	H	A	B	H	A	H	A	B	B	B	A	A	B	H	H	H	H	H	H	H	B	B	H	H	H	A	H	H	H	A	A	H	H	B	A	H	B	H	H	A	H	H	A	B	B	B	B	B				
ISU136	B	B	A	B	H	A	A	H	B	B	B	H	C	H	B	H	J	H	H	H	H	B	C	B	J	H	H	A	H	A	H	A	H	A	A	B	H	B	A	B	A	H	A	B	B	A	B	H	H	B			
PIO10.5	B	C	A	H	D	A	H	B	H	B	H	C	H	D	H	H	H	B	B	H	B	D	H	A	B	H	A	B	A	H	B	A	A	D	H	H	H	B	A	B	B	D	D	H	H	H	H	C	B	B			
ISU032B	B	B	A	B	H	H	A	B	H	H	H	H	B	-	H	H	H	H	B	B	H	B	H	A	B	A	B	A	H	A	B	H	C	A	A	H	H	H	H	C	A	C	A	H	H	H	H	A	H	B	B		
ISU156	B	B	A	B	H	H	A	B	H	H	H	H	H	H	H	H	H	H	B	B	H	B	H	A	B	B	H	A	B	B	H	H	H	H	A	B	A	B	B	A	B	B	A	B	H	H	A	B	H	B			
ISU034	H	B	A	H	H	H	B	H	H	H	H	H	H	H	H	H	B	H	B	H	B	A	H	B	A	B	B	H	B	H	A	A	B	H	H	H	A	B	B	A	B	H	H	A	H	A	B	H	B	H			
UMC114	H	B	A	H	H	H	B	H	H	H	H	H	H	H	H	H	H	B	H	B	H	B	A	H	B	H	H	B	B	H	H	B	H	A	B	B	A	B	H	A	H	A	H	A	H	H	H	H	H	H			
ISU110	H	D	A	D	B	A	H	B	H	D	B	A	-	H	D	D	B	H	B	H	A	H	-	D	H	B	H	H	B	B	H	H	H	A	A	B	A	B	B	A	B	B	A	H	H	H	A	H	A	H	A		
ISU077	D	D	D	D	B	D	D	B	D	D	B	D	-	D	D	D	B	D	B	B	B	D	D	-	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D

Table A2. (continued)

	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152					
BNL14.07	B	H	H	H	H	B	H	A	A	A	H	A	A	H	B	H	H	B	A	H	A	H	B	B	H	H	A	B	B	H	H	H	H	H	B	A	B	A	H	B	A	H	B	A	H	B	H	B	H						
ISU066	H	H	H	H	H	B	B	A	H	A	H	H	A	H	B	H	H	B	A	H	A	H	B	B	H	H	A	B	B	H	H	H	H	H	B	H	B	A	H	B	H	H	B	A	B	H	B	H	B	H					
ISU037	H	H	H	H	H	B	B	H	H	A	H	H	A	H	B	H	H	B	A	H	H	B	H	B	H	H	A	B	B	H	H	H	H	H	B	H	B	H	B	H	B	H	B	H	B	H	B	H	B	H					
ISU117	A	H	A	A	A	B	B	H	H	A	H	H	A	H	B	H	H	B	A	H	H	B	H	B	H	H	A	B	B	H	H	H	H	H	H	H	B	H	B	H	H	B	A	B	H	B	H	B	H						
ISU086	H	H	A	A	H	H	B	H	H	A	H	H	A	H	H	H	H	A	H	H	B	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H	A	B	H	A	B	A	B	H	-	-	-	B					
UMC139	H	H	A	A	H	H	B	H	H	H	H	H	A	B	H	H	H	A	A	H	B	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	B	H	A	B	H	A	A	B	H	H	B	H	B						
ISU094	H	H	A	H	H	B	A	H	H	H	H	A	B	H	H	H	H	A	A	H	B	H	H	A	B	H	H	A	B	H	H	H	H	H	H	H	B	H	A	B	H	H	H	H	B	H	B	H	B						
ISU151	B	H	A	H	H	A	A	H	H	H	B	B	A	B	H	H	H	B	A	A	H	B	H	H	B	H	H	A	H	A	H	H	H	H	H	B	H	A	B	H	H	H	B	H	B	H	B	H	B						
ISU071	H	H	A	B	H	A	A	H	B	H	B	B	B	B	H	B	H	B	A	A	H	B	B	H	H	B	A	A	B	A	H	H	H	H	H	H	B	H	A	B	H	H	H	H	B	H	B	H	B						
ISU130	H	H	A	B	H	H	A	H	B	A	B	B	B	A	B	B	B	A	A	H	B	B	B	H	H	A	A	B	H	H	A	A	H	H	H	B	B	A	B	A	H	B	H	B	H	H	B	B	H						
NPI400	H	H	H	B	H	H	H	B	A	B	B	B	B	A	B	H	B	A	A	H	B	B	B	B	H	B	H	A	B	B	H	A	A	B	H	B	B	B	H	A	B	A	H	H	H	A	H	A	H	H					
PIO20622	H	H	H	B	H	H	H	B	A	B	H	B	B	A	H	H	B	A	A	H	B	B	B	B	H	H	A	B	B	H	H	A	B	B	B	B	H	A	B	H	H	H	H	H	A	H	A	H	H	H					
ISU021	H	H	B	H	H	H	B	A	H	B	A	B	H	B	B	A	A	H	B	B	H	A	H	A	A	H	B	H	B	H	H	H	H	H	H	H	B	A	B	A	A	B	H	H	H	B	B	H	A	A					
ISU068	B	H	A	A	H	H	B	A	H	B	H	-	H	B	H	A	A	H	H	B	B	H	A	C	A	A	H	B	H	B	B	B	B	H	H	H	B	B	A	A	B	H	A	H	B	B	H	A	A						
BNL06.16	B	A	H	A	B	B	H	A	A	B	B	B	H	B	H	A	H	H	H	A	H	A	H	B	H	H	H	H	A	B	H	H	H	H	H	H	B	B	H	A	H	B	H	A	H	H	B	H	A						
NPI212	H	A	B	A	B	B	A	B	A	B	H	B	B	H	B	H	A	H	H	H	A	H	A	H	C	H	H	A	H	B	H	A	B	H	H	H	A	H	H	C	-	-	-	-	-	-	-	-	-	-	H	H	B	H	A
UMC093	B	H	B	A	B	B	A	B	H	H	B	H	H	A	A	H	B	H	A	A	H	H	A	H	A	B	H	B	B	H	B	B	B	B	B	B	B	A	H	H	H	H	B	B	B	H	H	B	H	A	A				
NPI426	B	H	B	H	H	B	A	H	H	H	B	H	H	A	A	H	B	H	H	-	A	H	-	-	H	H	B	H	B	H	B	B	H	B	B	A	H	B	-	-	-	-	-	-	-	-	-	-	H	B	B	H	A		
ISU057	B	D	D	D	D	B	D	D	D	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	B	D	D	D	D	D	D	D	D	B	D	D	D	D	D	D	D	D	D	D	D	B	D	D				
BNL13.05	B	H	A	H	H	B	A	H	H	B	B	H	B	H	B	H	A	A	H	H	H	B	H	A	A	H	H	B	B	H	H	H	H	H	A	B	A	H	B	B	B	B	B	B	B	B	B	B	B	B	H				
BNL09.11	B	H	A	A	H	B	A	A	H	H	B	H	B	H	B	H	A	A	H	H	B	B	H	H	H	H	H	B	H	H	H	H	H	A	B	A	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B			
ISU091	B	H	A	A	H	B	H	A	H	H	B	H	B	B	H	A	A	H	H	B	B	H	H	H	H	H	H	H	H	H	H	H	H	A	B	A	H	B	B	C	C	H	B	H	H	B	H	B	B						
BNL03.06	A	H	H	H	A	H	A	H	H	B	B	A	H	H	A	A	B	H	H	H	H	A	A	H	A	A	H	A	H	A	H	H	H	H	H	A	B	B	H	A	H	A	A	A	H	A	A	A	B	A	B				
ISU079	A	H	H	H	A	H	A	H	B	A	H	A	A	A	A	B	H	H	H	H	A	H	A	H	A	H	A	H	A	H	H	H	H	H	H	B	B	A	A	H	H	A	A	A	A	A	A	A	A	B	B				
ISU136	A	H	H	A	A	H	H	H	H	B	A	H	A	A	H	H	H	B	A	H	B	A	H	A	H	A	H	A	H	H	H	H	H	H	H	B	B	A	A	H	A	A	A	A	A	A	A	A	A	B	B				
PIO10.5	A	H	A	A	D	H	A	H	H	C	A	H	A	A	A	H	H	H	B	C	H	A	H	A	H	A	H	A	H	A	H	B	H	H	B	H	H	A	A	A	H	A	H	B	D	B	D	H	C	B	B				
ISU032B	A	H	H	A	A	H	A	H	H	A	H	A	H	A	H	A	H	A	H	C	H	B	H	C	A	H	A	H	A	H	H	H	H	H	H	H	H	H	A	H	A	B	A	H	B	A	B	H	H	C	B				
ISU156	A	H	H	A	B	A	A	A	B	H	A	H	A	H	H	H	A	H	B	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H	B	A	B	A	H	B	H	B	H	H	C	B	B						
ISU034	A	H	A	A	B	A	H	A	H	B	A	H	A	H	H	H	A	H	B	H	H	H	A	H	H	H	A	H	A	H	A	H	A	H	H	H	H	B	A	B	A	H	B	H	H	H	C	B	B	B					
UMC114	A	H	A	A	B	A	A	A	H	B	A	H	A	H	H	A	A	B	B	H	H	H	H	H	H	H	A	H	A	H	H	H	H	H	H	H	B	A	B	A	H	B	H	B	H	H	B	B	B						
ISU110	A	H	A	H	H	B	B	A	A	H	B	A	H	B	B	B	A	B	A	H	H	H	D	H	D	H	A	A	A	H	D	B	H	H	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B				
ISU077	D	D	D	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D					



Table A2. (continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	
ISU048	H	A	H	H	B	A	B	H	H	H	H	H	A	H	H	B	B	A	H	A	H	A	B	B	H	B	H	H	B	B	A	B	B	A	H	A	A	H	A	H	A	A	B	H	H	B	-	B	H			
ISU146	H	B	A	H	B	H	B	H	B	A	A	H	A	A	A	H	H	H	H	A	B	H	H	A	H	H	B	A	H	B	A	H	B	H	H	H	H	B	B	H	B	H	H	B	H	H	A	B	H			
ISU036A	B	B	A	H	B	H	B	H	B	H	A	H	H	A	H	H	B	A	H	H	H	H	B	H	H	A	A	H	B	H	A	B	H	H	H	H	H	B	B	B	H	B	H	B	H	H	A	H	A			
ISU125	A	B	A	H	B	B	H	H	B	H	H	B	H	A	H	H	B	A	B	B	H	H	H	A	H	A	A	H	A	H	A	C	A	H	H	H	H	B	B	B	B	H	B	H	H	C	A	-	H	H		
ISU145B	A	C	A	C	C	C	C	C	C	C	C	C	C	A	C	C	C	A	C	C	C	C	C	C	A	C	A	C	A	C	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	C	C			
NPI379	A	B	A	H	B	B	H	H	B	H	H	H	A	H	H	H	A	B	B	H	H	H	H	A	H	A	H	A	H	A	H	A	H	A	B	H	B	H	B	B	B	H	B	H	B	H	B	A	A	H	H	
UMC018	A	B	A	H	B	B	H	H	B	H	H	A	H	A	B	H	H	A	B	B	H	H	H	A	H	A	H	A	A	B	A	H	B	H	B	H	B	B	B	B	B	B	H	B	B	H	B	A	D	H	H	
ISU085B	A	H	A	H	B	B	H	B	H	H	A	H	A	B	H	H	A	A	B	H	B	H	H	H	A	A	A	B	D	H	B	H	A	B	H	B	B	B	B	B	B	B	B	H	H	B	A	A	B	H		
ISU024	H	H	H	B	H	B	A	A	H	B	A	B	B	H	H	H	H	A	B	H	B	H	A	A	A	A	A	H	A	H	A	H	A	B	A	H	A	H	H	A	H	B	H	B	A	A	H	A	H	-	H	H
ISU109	H	H	H	B	H	H	H	A	A	H	B	B	H	H	H	H	A	A	A	H	H	H	A	H	A	H	A	H	A	H	A	H	A	B	A	H	H	H	H	A	H	H	B	H	B	A	A	B	A	H		
ISU036B	A	H	H	B	H	H	H	A	A	H	H	B	B	A	H	H	H	A	A	H	B	A	H	H	A	H	A	H	A	A	H	A	B	A	H	H	B	H	H	H	A	B	H	B	H	A	B	H	H	B	A	H
ISU127	A	H	H	B	H	H	H	A	H	A	H	B	H	A	H	H	H	A	H	B	H	H	H	A	H	H	A	A	A	A	B	A	H	H	B	H	H	H	H	H	B	B	B	H	A	B	H	A	B	A	H	
ISU055	A	H	H	B	H	H	H	A	H	A	B	H	A	H	H	H	B	H	A	H	B	H	H	H	A	H	A	A	A	A	A	B	A	H	H	B	H	B	H	A	H	H	B	H	A	H	H	A	B	A	H	
UMC008	A	H	H	B	H	H	H	A	B	H	D	H	H	H	B	B	H	H	B	B	H	H	B	B	H	H	A	B	H	A	D	B	A	H	H	H	H	H	A	H	H	B	B	H	A	H	B	A	B	A	H	
ISU137	A	A	H	H	A	H	B	A	B	A	D	H	A	H	A	B	B	H	H	B	B	H	H	B	B	H	H	H	A	B	H	H	A	A	H	H	A	H	B	B	H	H	H	H	B	H	H	A	H			
ISU060A	D	A	H	H	D	D	D	B	B	D	D	D	H	D	D	D	D	B	B	B	H	D	B	B	D	D	D	D	D	D	D	D	D	B	B	H	H	A	H	A	D	B	B	B	H	A	H	D	B	D	A	D

Table A2. (continued)

	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102
ISU048	A	H	H	H	B	A	B	B	H	B	H	H	A	-	B	H	H	H	H	H	H	H	H	-	C	H	B	A	H	H	H	H	H	H	H	H	A	A	B	H	B	A	A	H	H	H	A	H	B	H	A
ISU146	H	H	A	B	B	A	B	B	H	B	H	H	H	B	A	B	H	H	H	A	A	H	A	H	H	H	H	B	A	H	H	H	H	B	B	H	H	A	A	H	A	B	B	A	B	H	H	B	A	B	H
ISU036A	A	B	H	H	B	A	B	B	H	B	H	A	B	H	B	B	B	B	H	A	A	H	A	H	B	H	B	A	H	H	H	H	B	B	H	H	A	A	A	H	A	B	B	A	B	H	H	A	B	H	A
ISU125	H	B	H	H	C	A	H	B	H	H	-	A	B	C	C	B	B	C	H	A	A	H	A	-	B	B	B	B	A	H	H	H	B	B	B	B	A	H	A	H	A	B	C	A	C	B	A	H	A	H	
ISU145B	C	C	C	C	C	C	C	C	C	A	C	C	-	C	C	C	C	C	A	C	C	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	C	A	C	A	C	A	C	A	C	A	C	A	
NPI379	H	B	H	H	H	H	H	H	H	H	A	H	B	A	B	H	B	A	H	H	H	A	H	-	H	H	B	B	A	H	H	H	H	H	H	B	H	H	A	H	A	B	H	A	H	B	A	A	A	A	H
UMC018	H	B	H	H	H	B	H	H	H	A	H	B	C	B	H	B	A	H	H	A	H	A	H	C	H	H	B	B	H	H	A	H	H	B	H	H	A	H	A	B	H	A	H	B	H	A	A	A	H		
ISU085B	H	B	H	H	B	B	A	H	H	B	H	B	H	B	H	H	A	H	H	A	H	H	A	H	B	H	H	B	H	H	A	H	H	B	A	H	H	A	H	H	B	A	A	H	H	A	H	H	A	H	
ISU024	A	B	H	H	H	B	H	H	H	-	H	H	-	H	-	-	B	A	B	A	H	-	-	B	H	H	B	B	H	B	H	B	H	B	H	B	A	B	A	H	A	B	B	A	H	B	H	H	B	B	
ISU109	B	B	H	B	H	C	B	B	A	H	B	H	B	H	H	A	A	B	A	B	A	H	A	B	H	B	B	H	B	H	H	H	B	B	A	B	A	B	A	B	A	B	B	A	H	B	H	H	B	B	
ISU036B	B	B	H	B	A	B	B	A	A	B	H	B	B	H	A	H	B	A	H	A	H	A	H	B	A	B	H	B	B	H	A	H	B	H	A	B	A	B	A	B	A	B	H	B	H	H	B	H	H	H	
ISU127	B	B	H	H	A	B	B	A	A	B	B	H	B	B	H	A	H	A	H	A	H	A	H	B	A	B	H	B	B	A	A	H	H	A	A	B	A	B	A	B	A	B	B	H	B	H	H	H	H	H	
ISU055	B	B	H	H	H	A	H	B	A	A	B	B	H	B	B	H	A	H	H	H	H	H	B	H	B	H	B	A	A	H	H	A	A	B	A	B	A	H	H	A	B	A	B	B	H	B	B	A	H	H	H
UMC008	B	B	H	H	A	H	H	H	B	B	C	B	H	A	A	H	A	H	B	B	B	B	B	B	B	B	B	A	A	H	H	A	B	A	H	H	A	B	H	B	B	H	B	H	A	H	A	H	H	A	
ISU137	B	B	A	H	H	A	H	H	B	H	B	B	H	A	A	H	A	H	A	H	B	A	B	B	B	B	B	A	A	H	H	A	B	A	H	H	A	B	A	H	A	B	B	H	H	H	H	H	H	H	
ISU060A	H	D	A	A	H	A	D	H	D	B	D	D	B	H	A	A	A	H	H	D	B	A	B	D	B	A	B	D	H	B	H	H	A	B	A	B	A	B	A	B	B	H	D	D	D	D	B	H	H		

Table A2. (continued)

	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152
ISU048	H	A	A	B	B	H	B	A	B	H	H	B	H	H	A	H	B	H	H	H	B	H	H	H	H	B	A	B	H	A	H	H	A	H	H	H	H	H	A	B	B	H	B	H	H	H	H			
ISU146	H	H	A	A	B	B	B	B	H	H	B	B	H	B	H	A	B	H	H	H	H	H	H	H	H	A	H	H	H	B	H	H	A	B	A	B	H	B	B	B	H	H	H	H	H	H	A	H	H	
ISU036A	H	B	A	A	H	B	B	H	B	A	B	B	H	B	H	A	B	A	H	H	A	B	H	H	A	H	H	A	H	B	B	H	H	A	B	B	H	H	H	A	B	H	H	H	H	A	B	A		
ISU125	H	B	A	A	H	B	B	H	C	H	B	H	H	B	H	A	B	A	H	H	H	B	H	-	-	C	C	A	H	B	C	B	H	H	A	B	B	H	A	B	H	H	H	-	-	C	C			
ISU145B	C	C	A	A	C	C	C	C	C	C	C	C	C	C	C	A	C	A	C	C	C	C	C	C	C	A	C	C	A	C	C	C	C	C	C	C	A	C	A	C	C	C	C	C	A	A	C	C		
NPI379	H	H	A	A	H	H	H	A	H	H	B	A	B	B	H	A	H	A	A	B	H	B	C	-	-	H	H	A	H	B	B	B	A	H	H	B	B	A	-	-	B	-	B	C	-	H	A	B	H	
UMC018	H	H	A	A	H	H	H	A	H	H	B	A	B	B	B	A	H	A	A	B	H	B	H	A	H	H	H	A	H	B	B	B	A	H	H	B	B	A	A	H	H	B	H	H	A	A	C	A		
ISU085B	H	H	A	A	H	H	H	A	H	H	B	H	B	B	B	A	H	A	A	B	A	B	H	A	H	H	A	H	H	B	B	A	A	H	A	H	H	B	A	A	H	H	B	A	H	D	-	-	A	
ISU024	A	B	B	B	B	A	B	A	A	A	H	H	H	A	H	H	H	B	H	H	H	B	A	H	H	H	H	H	H	H	H	H	B	H	H	B	B	A	H	H	H	B	A	H	H	H	A	H	B	
ISU109	A	B	B	H	B	H	B	A	A	A	H	H	B	A	H	H	H	B	H	H	A	B	A	H	H	H	H	H	H	H	H	H	H	H	H	B	B	A	H	H	B	A	A	H	H	H	H	H		
ISU036B	A	B	B	A	B	H	B	B	A	A	H	H	B	H	H	A	H	H	B	H	H	A	B	A	B	A	H	A	H	A	A	H	H	H	H	B	H	A	H	H	H	H	A	H	B	H	H	H		
ISU127	A	B	B	H	B	D	B	B	H	B	H	B	B	A	H	B	B	B	H	H	A	B	A	B	A	H	A	A	A	A	A	H	H	H	A	B	H	H	A	A	B	A	A	H	B	H	H	H		
ISU055	A	B	B	H	H	B	C	H	B	H	B	B	A	H	B	B	B	H	H	A	B	A	B	A	H	H	A	A	A	A	H	H	H	B	H	H	A	H	A	A	B	A	A	H	H	H	H	H		
UMC008	H	H	H	B	H	H	B	H	H	B	H	B	B	A	H	B	B	B	H	H	A	B	A	B	H	H	H	A	A	H	B	H	H	H	B	H	A	H	A	A	B	A	H	B	H	H	B	H	A	
ISU137	H	H	H	B	H	H	B	H	H	B	H	B	B	A	H	B	H	B	H	H	A	B	A	B	H	H	H	A	A	H	B	H	H	H	B	A	B	H	H	A	A	B	B	H	B	H	H	H	A	
ISU060A	H	H	H	B	H	A	B	H	A	B	A	B	A	A	H	H	H	H	H	H	A	B	A	A	H	H	H	H	A	A	H	B	D	D	H	B	A	D	D	D	H	A	B	H	B	B	D	H	H	A

Table A3. Plot means and adjusted entry means for morphological traits.

Pedigree	Entry #	Rep	Plot	Blk	Plant height		Plant height to the uppermost node		Peduncle length		Panicle length		Panicle width	
					exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	1	1	5	10	309.6	299.6	222.8	217.25	86.8	82.6	31.6	36	31.6	32.08
		2	153	17	303.8		225.4		78.4		40.4		32.5	
CK60 x PI229828-F2:3	2	1	100	2	304.7	297.1	247	241.45	57.7	56.2	24	23.7	24	22.74
		2	125	19	289		234.3		54.7		23.4		21.6	
CK60 x PI229828-F2:3	3	1	143	13	315.6	316.8	248.4	249.71	67.2	67	25.9	26.85	22.7	23.51
		2	110	20	314		247.3		66.7		27.8		24.4	
CK60 x PI229828-F2:3	4	1	107	2	249.5	233.5	190.1	174.48	59.4	59.6	21.6	21.1	19	19.22
		2	57	23	215.5		155.7		59.8		20.6		19.5	
CK60 x PI229828-F2:3	5	1	95	11	232.4	238.2	173.7	177.12	58.7	60.6	31.9	31.85	40.2	37.84
		2	99	14	246.5		184.1		62.4		31.8		35.4	
CK60 x PI229828-F2:3	6	1	23	8	268.9	246.7	191	175.77	77.9	72.3	34.9	35.05	34.5	32.99
		2	82	16	234.9		168.2		66.7		35.2		31.5	
CK60 x PI229828-F2:3	7	1	35	12	278.4	296.2	231.5	248.97	46.9	47.3	25.1	25.15	23.3	23.66
		2	75	16	321.1		273.4		47.7		25.2		24	
CK60 x PI229828-F2:3	8	1	43	6	297.7	292	224.3	220.96	73.4	70.4	21	23.3	20.5	21.97
		2	14	21	287.3		220		67.3		25.6		23.5	
CK60 x PI229828-F2:3	9	1	40	6	283	270.3	219	206.61	64	64.4	32.6	32.65	27.9	29.31
		2	106	14	270.3		205.5		64.8		32.7		30.7	
CK60 x PI229828-F2:3	10	1	71	4	336.3	328.4	262.2	257.53	74.1	71.2	31.8	29.85	28.2	27.6
		2	109	20	321.2		252.9		68.3		27.9		27.1	
CK60 x PI229828-F2:3	11	1	84	5	285.7	278.1	214.6	206.59	71.1	70.5	28.5	30.05	26.5	28.48
		2	120	20	263.1		193.3		69.8		31.6		30.5	
CK60 x PI229828-F2:3	12	1	92	11	294	299	227.5	228.12	66.5	69.4	25.7	27.1	25.6	26.38
		2	149	17	306.1		233.9		72.2		28.5		27.1	
CK60 x PI229828-F2:3	13	1	41	6	330.1	308.4	253.8	233.26	76.3	75	33.3	32.65	33.7	32.43
		2	134	22	302.4		228.8		73.6		32		31.2	
CK60 x PI229828-F2:3	14	1	154	3	236.1	241.1	178.9	186.14	57.2	55.3	18.1	18.85	17.8	19.44
		2	104	14	240.5		187.2		53.3		19.6		21	
CK60 x PI229828-F2:3	15	1	7	10	294.5	273.8	222.2	203.96	72.3	71	28.8	28.75	27.4	28.87
		2	74	16	270		200.4		69.6		28.7		30.3	
CK60 x PI229828-F2:3	16	1	130	1	299.2	256.9	223.5	181.21	75.7	76.2	34	32.85	32.8	33.67
		2	130	19	217.6		141		76.6		31.7		34.6	
CK60 x PI229828-F2:3	17	1	115	9	293.5	279.2	222.6	206.06	70.9	72.8	34.3	33.55	30.7	31.2
		2	111	20	262.7		188		74.7		32.8		31.7	
CK60 x PI229828-F2:3	18	1	78	5	299.8	297	236.5	233.13	63.3	63.2	31.7	31.75	39.2	38.36
		2	61	24	286.8		223.7		63.1		31.8		37.5	
CK60 x PI229828-F2:3	19	1	11	10	331.8	307.5	269	252.75	62.8	55.2	27.7	28.25	23	23.5
		2	135	22	300.9		253.4		47.5		28.8		24	
CK60 x PI229828-F2:3	20	1	101	2	297.3	287	231.7	224.17	65.6	63.5	28.5	26.9	25.1	22.97
		2	98	14	284.4		223		61.4		25.3		20.8	
CK60 x PI229828-F2:3	21	1	88	11	325.6	328.9	256.8	260.62	68.8	66.7	31.9	30.9	32.5	29.95
		2	92	28	327.8		263.2		64.6		29.9		27.3	
CK60 x PI229828-F2:3	22	1	150	3	283.1	294.6	208.8	220.93	74.3	73	31.1	30.8	32.5	33.92
		2	141	22	303.4		231.7		71.7		30.5		35.3	
CK60 x PI229828-F2:3	23	1	93	11	323	300.8	255.4	232.97	67.6	65.9	31.1	32.7	27.3	28.25
		2	13	21	269.4		205.2		64.2		34.3		29.2	
CK60 x PI229828-F2:3	24	1	59	7	114.2	114.6	48.4	50.75	65.8	64	26.8	26.65	27.1	27.44
		2	136	22	122.6		60.5		62.1		26.5		27.8	

Table A3. (continued)

Pedigree	Entry			Number of nodes			Leaf length		Leaf width		Number of tillers		Stalk circumference		GDD to anthesis	
	#	Rep	Plot	Blk	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	1	1	5	10	11.2	11	70.7	73.3	6.3	6.3	0.6	0.9	6.4	6.39	1172	1175.9
		2	153	17	11.1		75.9		6.3		1		6.5		1172	
CK60 x PI229828-F2:3	2	1	100	2	12.4	12.1	74.6	77.3	5.8	6.1	1.5	2.4	4.9	4.93	1318	1284.9
		2	125	19	11.9		80.1		6.4		3.2		5.3		1246	
CK60 x PI229828-F2:3	3	1	143	13	11.3	11.2	73.6	77.1	6.3	6.2	1.7	1.4	5	4.67	1200	1179.6
		2	110	20	10.9		80.5		6.1		1.3		4.6		1172	
CK60 x PI229828-F2:3	4	1	107	2	9.9	9.67	79.8	80.7	5.8	6	2.1	2.3	5	4.55	1112	1096.3
		2	57	23	9.3		81.8		6.1		2.6		3.8		1085	
CK60 x PI229828-F2:3	5	1	95	11	12.9	11.9	85	85.8	5.8	6	0.9	1	5.7	5.32	1375	1360.4
		2	99	14	10.9		86.7		6.1		1.1		4.9		1351	
CK60 x PI229828-F2:3	6	1	23	8	10.3	10.3	75	75.8	5.9	6.1	0.7	0.6	5.5	5.38	1085	1096.6
		2	82	16	10.4		76.6		6.2		0.5		5.5		1085	
CK60 x PI229828-F2:3	7	1	35	12	13.1	13.3	73.3	71.3	6.7	6.9	0.1	0.5	5.2	5.19	1351	1350.9
		2	75	16	13.6		69.3		7.1		0.8		5.5		1334	
CK60 x PI229828-F2:3	8	1	43	6	11.1	11.3	68.2	70	6.7	6.6	1.5	1.4	5.9	5.37	1217	1198.2
		2	14	21	11.5		71.6		6.4		1.2		4.9		1172	
CK60 x PI229828-F2:3	9	1	40	6	11.7	11.9	69.4	70.3	7	7.1	0.7	0.7	5.9	5.71	1200	1203.4
		2	106	14	11.9		71		7.1		0.7		5.8		1200	
CK60 x PI229828-F2:3	10	1	71	4	12.5	11.9	82.5	82	5.1	5.5	1.8	2.6	4.7	4.64	1246	1250.5
		2	109	20	11.2		81.4		5.8		3.3		4.8		1246	
CK60 x PI229828-F2:3	11	1	84	5	10.5	10.5	78.6	77.3	6.6	6.8	0.8	0.7	4.7	4.51	1162	1104.6
		2	120	20	10.3		76.1		6.9		0.7		4.4		1057	
CK60 x PI229828-F2:3	12	1	92	11	11.8	11.5	70	72.5	6.1	6.6	1.1	1.4	4.7	4.87	1162	1123
		2	149	17	11.4		75.1		7.1		1.6		5		1085	
CK60 x PI229828-F2:3	13	1	41	6	12.4	12.5	79.4	81.7	6.9	6.9	0.5	0.9	5.7	5.29	1246	1244.3
		2	134	22	12.6		83.9		6.9		1.2		5.2		1233	
CK60 x PI229828-F2:3	14	1	154	3	9.8	9.66	68.2	69.9	6.4	6.6	1.8	1.8	5	5.17	1112	1123.5
		2	104	14	9.4		71.5		6.7		1.8		5.3		1137	
CK60 x PI229828-F2:3	15	1	7	10	10.7	10.6	77.2	77.2	5.9	6.3	0.3	1.1	5	4.75	1112	1118.9
		2	74	16	10.6		77		6.6		1.8		4.7		1112	
CK60 x PI229828-F2:3	16	1	130	1	11.4	11.3	83.2	83.5	5.8	6.1	1.3	1.3	5.7	5.53	1217	1196
		2	130	19	11.2		83.8		6.4		1.2		5.4		1172	
CK60 x PI229828-F2:3	17	1	115	9	9.6	9.7	81.5	81.1	6.1	6.7	1.1	1.3	4.6	4.48	1057	1045.7
		2	111	20	9.6		80.6		7.2		1.6		4.5		1040	
CK60 x PI229828-F2:3	18	1	78	5	12	11.9	76	73.9	5.4	5.6	0.8	1.4	4.7	4.7	1217	1216.4
		2	61	24	11.9		71.8		5.7		1.9		4.5		1217	
CK60 x PI229828-F2:3	19	1	11	10	11.7	11.6	72.2	74.8	6.7	7.3	1.2	1.3	5.7	5.36	1172	1175.1
		2	135	22	11.6		77.3		7.9		1.1		5.2		1172	
CK60 x PI229828-F2:3	20	1	101	2	10.5	10.6	75	73.5	6.2	6.4	1	1.4	4.5	4.37	1162	1155.6
		2	98	14	10.7		72		6.5		1.7		4.4		1152	
CK60 x PI229828-F2:3	21	1	88	11	10.6	10.8	85.1	83.7	7.7	7.4	1	0.9	5.1	5.13	1217	1219.8
		2	92	26	11.2		82.4		7.1		1		5.1		1233	
CK60 x PI229828-F2:3	22	1	150	3	10.9	10.9	74.6	78.1	7.1	7.2	1.8	1.5	5.5	5.44	1152	1118.9
		2	141	22	10.9		81.4		7.3		1.1		5.4		1085	
CK60 x PI229828-F2:3	23	1	93	11	12.1	11.8	75.9	76.8	5.8	6	2	2.1	4.5	4.83	1152	1145.7
		2	13	21	11.7		77.7		6.1		2.1		4.9		1152	
CK60 x PI229828-F2:3	24	1	59	7	11.3	11.8	68.4	68.9	5.7	6.8	0.5	0.7	7.2	7.1	1246	1229.4
		2	136	22	12.3		69.5		7.8		0.7		7.2		1200	

Table A3. (continued)

Pedigree	Entry #	Rep	Plot	Blk	Plant height		Plant height to the uppermost node		Peduncle length		Panicle length		Panicle width	
					exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	25	1	121	1	287.2	252.1	220.3	188.44	66.9	64.1	29.7	29.4	30.9	31.2
		2	55	23	218.4		157.1		61.3		29.1		31.5	
CK60 x PI229828-F2:3	26	1	114	9	300.9	300.6	232.4	232.52	68.5	68	26.4	28.85	22.8	24.56
		2	34	25	298.3		230.8		67.5		31.3		26.2	
CK60 x PI229828-F2:3	27	1	90	11	278.6	288.2	233.3	237.23	45.2	50.2	23.4	24.35	21.4	22.68
		2	73	16	302.5		247.3		55.2		25.3		23.9	
CK60 x PI229828-F2:3	28	1	133	13	256.7	261.6	183.7	193.91	73	67.4	31.2	30.45	26.3	26.03
		2	156	17	268.7		207		61.7		29.7		25.7	
CK60 x PI229828-F2:3	29	1	134	13	279.4	285	196.7	206.41	82.7	79.3	32.3	33.1	38.2	39.28
		2	100	14	293.3		217.4		75.9		33.9		40.3	
CK60 x PI229828-F2:3	30	1	69	4	291.7	278.6	220.7	215.55	71	63.5	23	23.3	20	20.79
		2	12	18	255.6		199.6		56		23.6		21.7	
CK60 x PI229828-F2:3	31	1	85	11	298.7	287.7	236.3	221.18	62.4	65.6	25	25.85	19.4	19.93
		2	42	15	273.7		205		68.7		26.7		20.4	
CK60 x PI229828-F2:3	32	1	6	10	320.1	292.9	246.1	222.45	74	70.7	32.3	29.55	34	29.94
		2	87	26	273.4		206.1		67.3		26.8		25.8	
CK60 x PI229828-F2:3	33	1	111	9	347.9	344.8	284.3	282.42	63.6	62.4	31.4	31.1	26	25.46
		2	43	15	340.7		279.4		61.2		30.8		24.8	
CK60 x PI229828-F2:3	34	1	37	6	286.5	273.3	202.7	188.1	83.8	85.5	39.1	38.7	42.9	41.62
		2	68	24	266.2		179		87.2		38.3		40.4	
CK60 x PI229828-F2:3	35	1	91	11	323.4	306.6	255.9	240.38	67.5	65.1	32.6	32.8	31.6	30.93
		2	35	25	285.8		223.1		62.7		33		30.2	
CK60 x PI229828-F2:3	36	1	117	9	294.9	285.2	230.9	218.92	64	66	31.4	32	28.5	31.19
		2	9	18	262.5		194.6		67.9		32.6		33.9	
CK60 x PI229828-F2:3	37	1	79	5	335.2	342.8	263.6	265.85	71.6	75.7	32.2	34.45	26.8	29.81
		2	89	26	342.7		263		79.7		36.7		32.7	
CK60 x PI229828-F2:3	38	1	39	6	289.2	263.3	227.7	196.78	61.5	67.2	25.8	27.2	24.9	26.11
		2	60	23	240.4		167.6		72.8		28.6		27.4	
CK60 x PI229828-F2:3	39	1	75	5	281	274.8	218.2	213	62.8	61.6	25.5	24.85	25.9	24.35
		2	103	14	267.8		207.5		60.3		24.2		22.7	
CK60 x PI229828-F2:3	40	1	18	8	299.7	279.6	223.4	210.82	76.3	69.6	24.5	24.8	25.5	26.92
		2	10	18	250.3		187.5		62.8		25.1		28.5	
CK60 x PI229828-F2:3	41	1	64	4	337.3	312.6	257.4	234.09	79.9	79.8	31.7	32	29.7	30.57
		2	101	14	295.5		215.8		79.7		32.3		31.4	
CK60 x PI229828-F2:3	42	1	13	8	295.9	291.1	228.7	224.46	67.2	68.2	26.2	25.4	23.2	22.35
		2	108	14	294.5		225.3		69.2		24.6		21.5	
CK60 x PI229828-F2:3	43	1	25	12	332.6	323.4	252	243.85	80.6	78.9	24.9	25.55	20.2	19.74
		2	133	22	322.1		244.9		77.2		26.2		19.3	
CK60 x PI229828-F2:3	44	1	77	5	325.6	330.8	262.4	268.29	63.2	61.4	25.9	26.95	19.3	20.52
		2	137	22	338.2		278.7		59.5		28		21.7	
CK60 x PI229828-F2:3	45	1	53	7	275.6	266.5	210	203.8	65.6	62.3	25.5	25.1	23.9	24.18
		2	19	21	250.3		191.3		59		24.7		24.5	
CK60 x PI229828-F2:3	46	1	38	6	239.3	192.5	183.4	136.91	55.9	55.3	26.4	26.45	29.6	29.51
		2	148	17	157.9		103.3		54.6		26.5		29.4	
CK60 x PI229828-F2:3	47	1	129	1	300.1	297.3	237.2	232.43	62.9	64.3	36.9	36.15	30.2	31.61
		2	88	26	298.6		232.9		65.7		35.4		32.9	
CK60 x PI229828-F2:3	48	1	118	9	301.4	309.5	234.9	242.16	66.5	66.8	26.3	26.8	25.6	26.88
		2	93	26	315.2		248.1		67.1		27.3		28	

Table A3. (continued)

Pedigree	Entry			Number of nodes		Leaf length		Leaf width		Number of tillers		Stalk circumference		GDD to anthesis		
	#	Rep	Plot	Blk	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	25	1	121	1	11.4	11.7	73.6	73.8	5.7	6.1	1.4	1.4	5.3	5.51	1217	1213.4
		2	55	23	11.9		74.1		6.4		1.5		5.1		1217	
CK60 x PI229828-F2:3	26	1	114	9	10.9	11.2	71.4	73.1	6.5	6.7	1	1.5	5	5.09	1172	1175.3
		2	34	25	11.4		74.8		6.9		2.1		4.9		1200	
CK60 x PI229828-F2:3	27	1	90	11	10.5	10.8	84.6	84	6.3	6.5	1.5	1.3	4.8	4.58	1318	1290.1
		2	73	16	11.2		83.3		6.7		1.1		4.4		1257	
CK60 x PI229828-F2:3	28	1	133	13	10.4	10.5	75.7	74.6	6.6	6.7	1.2	1.6	5.9	5.63	1152	1132.5
		2	156	17	10.8		73.6		6.7		2		5.5		1125	
CK60 x PI229828-F2:3	29	1	134	13	10.5	10.7	81.9	81.4	6	6.3	1	1.2	5.3	5.08	1152	1143.8
		2	100	14	10.8		80.8		6.6		1.4		5		1152	
CK60 x PI229828-F2:3	30	1	69	4	10.7	11.1	72.1	72	6.1	6.1	0.6	0.6	4.9	4.76	1172	1170.2
		2	12	18	11.4		71.9		6.1		0.7		4.7		1172	
CK60 x PI229828-F2:3	31	1	85	11	10.6	10.6	72.5	72.4	6.2	6.6	1.2	1.2	4.5	4.61	1200	1186.4
		2	42	15	10.6		72.5		6.9		1.3		4.3		1184	
CK60 x PI229828-F2:3	32	1	6	10	12.6	12.3	78.7	81.2	6.1	6.2	0.9	0.9	5.2	5.09	1246	1245.1
		2	87	26	12.2		83.6		6.2		1		5.1		1246	
CK60 x PI229828-F2:3	33	1	111	9	13.5	12.8	83.1	81.6	6.3	6.4	2.2	2.4	4.4	4.57	1351	1335
		2	43	15	12		80.3		6.4		2.7		4.4		1334	
CK60 x PI229828-F2:3	34	1	37	6	10.8	10.9	73.5	76.9	6.8	7.2	0.8	0.9	5.8	5.75	1152	1148
		2	68	24	11		80.2		7.5		1.1		5.8		1125	
CK60 x PI229828-F2:3	35	1	91	11	11.3	11.2	78.1	78.2	6.5	6.6	0.7	1.1	5.3	5.28	1172	1163.7
		2	35	25	11.1		78.3		6.6		1.4		4.9		1172	
CK60 x PI229828-F2:3	36	1	117	9	12.2	12.3	74	74.7	6.7	7	2	1.7	5.3	5.31	1217	1208
		2	9	18	12.4		75.3		7.2		1.6		5.3		1217	
CK60 x PI229828-F2:3	37	1	79	5	11.1	11.2	75.8	78	6.1	6.5	0.8	1.8	4.5	5.01	1162	1127.8
		2	89	26	11.4		80.3		6.9		2.8		5.5		1112	
CK60 x PI229828-F2:3	38	1	39	6	11.7	11.2	72.1	74.5	7.1	7	1.7	1.4	5.4	4.9	1162	1175.6
		2	60	23	10.5		76.7		6.9		1.1		4.2		1184	
CK60 x PI229828-F2:3	39	1	75	5	11.8	11.5	77.1	74.6	6.2	6.6	2	1.8	4.9	4.76	1200	1149.4
		2	103	14	11.3		72.1		6.9		1.4		4.6		1112	
CK60 x PI229828-F2:3	40	1	16	8	10.4	10	77.6	74.5	5.9	5.9	1.9	2.1	5	4.59	1085	1086.9
		2	10	18	9.6		71.3		5.9		2.5		4.3		1085	
CK60 x PI229828-F2:3	41	1	64	4	11.4	11.5	80	83.8	6	6.2	0.9	1.2	5	4.94	1246	1260.2
		2	101	14	11.6		87.6		6.3		1.3		5		1269	
CK60 x PI229828-F2:3	42	1	13	8	10	10.2	70.2	70.3	7	7	2.9	2.1	4.7	4.47	1152	1158.4
		2	108	14	10.3		70.4		6.9		1.3		4.4		1152	
CK60 x PI229828-F2:3	43	1	25	12	11.6	11.2	71.4	73	5.3	5.6	0.4	0.5	4.9	4.81	1152	1156.6
		2	133	22	10.9		74.7		5.9		0.4		5		1152	
CK60 x PI229828-F2:3	44	1	77	5	12.8	12.9	71.7	72.3	6.8	6.9	0.6	0.9	4.8	5.03	1334	1311.7
		2	137	22	13.2		72.9		6.9		1		5.3		1300	
CK60 x PI229828-F2:3	45	1	53	7	10.8	11.3	73.7	70.9	7	7.3	1.5	1.2	5.4	5.18	1152	1143.9
		2	19	21	11.7		68.1		7.5		0.6		4.9		1125	
CK60 x PI229828-F2:3	46	1	38	6	11.2	11.4	77.3	79.2	5.5	5.6	1.6	1.1	5.7	5.26	1217	1222.6
		2	148	17	11.6		81		5.7		0.5		5.1		1217	
CK60 x PI229828-F2:3	47	1	129	1	11.5	11.7	77.2	75.1	7.4	7.4	2.2	1.9	5.5	5.47	1172	1156.6
		2	88	26	11.9		73.1		7.3		1.7		5.3		1152	
CK60 x PI229828-F2:3	48	1	118	9	11.8	11.7	69.5	71.6	6.8	7	1.8	1.7	6.2	5.74	1200	1178.9
		2	93	26	11.6		73.8		7.2		1.7		5.3		1172	

Table A3. (continued)

Pedigree	Entry #	Rep	Plot	Blk	Plant height		Plant height to the uppermost node		Peduncle length		Panicle length		Panicle width	
					exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	49	1	36	12	299.1	293.9	242.4	240.05	56.7	52.7	24.6	23.15	22	19.58
		2	22	21	281.9		233.2		48.7		21.7		17.2	
CK60 x PI229828-F2:3	50	1	132	1	269.6	268	202.1	200.88	67.5	66.2	29.9	30.65	28.2	30.21
		2	24	21	265.7		200.8		64.9		31.4		32.2	
CK60 x PI229828-F2:3	51	1	31	12	298.7	291.4	232.6	220.31	66.1	71.4	27.2	29.25	27.1	28.62
		2	97	14	289		212.4		76.6		31.3		30.1	
CK60 x PI229828-F2:3	52	1	139	13	360.2	349.7	278	273.17	82.2	77.1	36.7	34.85	32.9	30.98
		2	80	16	344.1		272.2		71.9		33		29	
CK60 x PI229828-F2:3	53	1	1	10	340.2	334.2	263.1	262.2	77.1	72.4	35.1	35.1	33.7	34.21
		2	116	20	336		268.4		67.6		35.1		34.8	
CK60 x PI229828-F2:3	54	1	46	6	314.5	283	241	213.82	73.5	69.1	30.1	30.8	29.4	30.13
		2	7	18	246.7		182.1		64.6		31.5		31	
CK60 x PI229828-F2:3	55	1	81	5	316	318.5	255.4	253.7	60.6	64.2	27.5	28.35	24.5	25.24
		2	48	15	314.8		247.1		67.7		29.2		25.9	
CK60 x PI229828-F2:3	56	1	109	9	330.8	326.9	264.5	265.52	66.3	61.8	28.8	29.25	27.6	27.57
		2	50	23	317.8		260.5		57.3		29.7		27.5	
CK60 x PI229828-F2:3	57	1	42	6	316.4	304.8	226	221.07	90.4	84.2	34.3	31.35	34.6	32.15
		2	78	16	307.9		230		77.9		28.4		29.7	
CK60 x PI229828-F2:3	58	1	54	7	263.7	245	192.5	173.4	71.2	71.6	20.9	20.5	17.4	17.66
		2	150	17	230.4		158.5		71.9		20.1		17.9	
CK60 x PI229828-F2:3	59	1	57	7	335.5	329	266.6	263.81	68.9	65.7	29.1	29.25	27.5	27.4
		2	45	15	321.4		259		62.4		29.4		27.3	
CK60 x PI229828-F2:3	60	1	108	2	288.9	281.5	222.4	212.78	66.5	68.5	32.5	32.9	27.7	28.29
		2	4	18	264.2		193.7		70.5		33.3		29	
CK60 x PI229828-F2:3	61	1	61	4	335.9	331.1	252.3	255.83	83.6	75.1	36	34.5	34	32.99
		2	15	21	322.1		255.5		66.6		33		32	
CK60 x PI229828-F2:3	62	1	47	6	277.8	259.4	196.9	186.87	80.9	72.8	31.2	31.6	27.9	28.15
		2	37	15	248.1		183.5		64.6		32		28.4	
CK60 x PI229828-F2:3	63	1	152	3	251.7	273	191.8	213.54	59.9	58.8	25.7	25.6	21.6	21.84
		2	147	17	288.3		230.7		57.6		25.5		22	
CK60 x PI229828-F2:3	64	1	73	5	300.6	303.4	240.1	243.08	60.5	60.1	26.9	27.15	25.2	26.27
		2	129	19	297.4		237.8		59.6		27.4		27.4	
CK60 x PI229828-F2:3	65	1	102	2	346.7	348.9	283.7	285.03	63	64.3	35.5	35.65	31.2	31.11
		2	77	16	360.9		295.3		65.6		35.8		31	
CK60 x PI229828-F2:3	66	1	153	3	208.6	213.7	149.3	154.83	59.3	57.8	26.8	26.4	26.2	26.76
		2	20	21	201.5		145.3		56.2		26		27.3	
CK60 x PI229828-F2:3	67	1	89	11	319.9	315.5	261	254.03	58.9	60.1	31.9	32.1	33.2	34.41
		2	8	18	296.1		234.8		61.3		32.3		35.7	
CK60 x PI229828-F2:3	68	1	99	2	313.2	304.4	243.7	235.37	69.5	68.6	28.6	28.7	23.7	24.43
		2	85	26	296.3		228.6		67.7		28.8		25.1	
CK60 x PI229828-F2:3	69	1	2	10	306.9	281	246.7	221.49	60.2	60.8	29.9	30.2	27.4	29.6
		2	123	19	261.8		200.4		61.4		30.5		31.9	
CK60 x PI229828-F2:3	70	1	12	10	272.5	243.2	198.7	173.97	73.9	69.7	28.9	27.45	27.4	28.6
		2	3	18	210.9		145.5		65.4		26		29.9	
CK60 x PI229828-F2:3	71	1	76	5	267.9	292	196.9	221.4	71	69.4	25.2	26.1	31	31.05
		2	154	17	314.9		247.2		67.7		27		31	
CK60 x PI229828-F2:3	72	1	116	9	327.9	309.5	247.5	230.36	80.4	78.3	34.1	31.75	31.8	30.58
		2	16	21	284		207.8		76.2		29.4		29.3	



Table A3. (continued)

Pedigree	Entry #	Number of nodes				Leaf length		Leaf width		Number of tillers		Stalk circumference		GDD to anthesis		
		Rep	Plot	Blk	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	49	1	36	12	10.7	11	67.1	66.7	6.6	6.9	0.8	1	5.1	4.69	1217	1220.5
		2	22	21	11.4		66.3		7.1		1		4.3		1217	
CK60 x PI229828-F2:3	50	1	132	1	10.5	10.9	75.5	75.6	6	6.4	1.5	1.9	5.1	5.13	1200	1214
		2	24	21	11.3		75.8		6.7		2.1		4.8		1233	
CK60 x PI229828-F2:3	51	1	31	12	11.3	11.2	78.4	77.9	7.3	7.4	1.4	1.9	5.1	4.93	1112	1135.2
		2	97	14	11		77.4		7.5		2.2		5		1152	
CK60 x PI229828-F2:3	52	1	139	13	11.3	12	79.3	78.4	7.1	7	1.4	1.4	5	5.14	1217	1205.5
		2	80	16	12.8		77.5		6.8		1.5		5.5		1200	
CK60 x PI229828-F2:3	53	1	1	10	13.2	12.9	74.2	74.1	7	7.2	0.7	1.3	5.2	5.08	1257	1255
		2	116	20	12.4		73.9		7.3		1.9		5.2		1246	
CK60 x PI229828-F2:3	54	1	46	6	10.7	10.7	71.5	72.3	6.3	6.3	1	1.6	5.1	4.88	1112	1130.9
		2	7	18	10.5		72.9		6.3		2.4		4.9		1152	
CK60 x PI229828-F2:3	55	1	81	5	12.8	12.6	74.4	77	6	6	2.2	2.5	4.6	4.49	1217	1233.4
		2	48	15	12.5		79.7		5.9		2.8		4		1269	
CK60 x PI229828-F2:3	56	1	109	9	11.9	12.1	82.3	79.5	5.9	5.8	1.8	1.8	5.1	4.88	1246	1259.7
		2	50	23	12.2		76.7		5.7		1.9		4.2		1284	
CK60 x PI229828-F2:3	57	1	42	6	9.8	9.95	79.7	81.1	6.7	7	1.4	1	5.2	4.57	1152	1140.6
		2	78	16	10.1		82.3		7.2		0.7		4.3		1112	
CK60 x PI229828-F2:3	58	1	54	7	9.2	9.35	73.9	77.3	6.3	6.5	0.6	1.1	5.3	5.07	1152	1159.2
		2	150	17	9.5		80.8		6.6		1.5		5		1152	
CK60 x PI229828-F2:3	59	1	57	7	11.6	11.7	68.8	70.5	7	7.1	1	1.4	5.2	4.91	1246	1259.6
		2	45	15	11.5		72.4		7.1		1.7		4.4		1269	
CK60 x PI229828-F2:3	60	1	108	2	11.3	11.2	76.1	75.7	6.3	6.5	1.3	1.6	5	4.84	1162	1175.1
		2	4	18	11		75.3		6.6		2		4.8		1200	
CK60 x PI229828-F2:3	61	1	61	4	11.4	11.4	76.4	78.1	5.9	6.1	2.2	1.7	5.5	5.1	1162	1184
		2	15	21	11.4		79.9		6.3		0.9		4.6		1200	
CK60 x PI229828-F2:3	62	1	47	6	11	11.2	71.7	72.8	6.8	7.1	1.1	1.2	6.1	5.64	1152	1199.4
		2	37	15	11.3		73.9		7.3		1.3		5.1		1246	
CK60 x PI229828-F2:3	63	1	152	3	10.7	11.2	61.8	63.6	6.8	7	1.9	1.8	4.5	4.62	1162	1163.2
		2	147	17	11.7		65.4		7.1		1.7		4.7		1162	
CK60 x PI229828-F2:3	64	1	73	5	11.6	11.4	71.5	69.4	7	6.8	1	1	4.9	4.77	1152	1149.7
		2	129	19	11.3		67.3		6.5		0.8		4.8		1152	
CK60 x PI229828-F2:3	65	1	102	2	12.6	12.9	77.3	77.7	6.6	6.5	0.8	1.2	5.6	5.18	1318	1304.8
		2	77	16	13.2		78.2		6.4		1.5		5		1284	
CK60 x PI229828-F2:3	66	1	153	3	10.4	10.9	72.6	74.7	6.6	6.6	2	1.8	5.5	5.52	1200	1207.9
		2	20	21	11.3		76.7		6.6		1.4		5.3		1217	
CK60 x PI229828-F2:3	67	1	89	11	12.1	11.3	82.2	81.9	6.5	6.4	2.4	2.1	5.7	5.29	1269	1250.4
		2	8	18	10.6		81.5		6.3		1.9		4.8		1246	
CK60 x PI229828-F2:3	68	1	99	2	11.7	11.7	73.9	73.3	7.5	7.4	0.7	0.9	5.2	5.02	1217	1204.5
		2	85	26	11.7		72.7		7.2		1.2		5		1200	
CK60 x PI229828-F2:3	69	1	2	10	11.3	11.2	77.6	78.9	6.9	7.1	0.8	0.8	6	5.95	1200	1214.5
		2	123	19	11.3		80.1		7.3		0.5		6.2		1217	
CK60 x PI229828-F2:3	70	1	12	10	10.1	9.94	76.4	75.1	7.4	7.4	1.6	2.1	4.8	5.01	1085	1115.7
		2	3	18	9.8		73.5		7.3		2.7		5.3		1152	
CK60 x PI229828-F2:3	71	1	76	5	11.4	11.3	76	77.3	6.8	7	1.1	0.8	5.9	5.66	1200	1176.5
		2	154	17	11.4		78.7		7.1		0.4		5.4		1162	
CK60 x PI229828-F2:3	72	1	116	9	11.1	10.9	84.1	85.3	5.8	6.3	2.4	2	5.1	5.04	1200	1181.8
		2	16	21	10.8		86.5		6.8		1.5		4.8		1172	

Table A3. (continued)

Pedigree	Entry #	Rep	Plot	Blk	Plant height		Plant height to the uppermost node		Peduncle length		Panicle length		Panicle width	
					exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	73	1	94	11	314	319.1	239.2	240.95	74.8	77.7	26.2	28.6	26.2	29.51
		2	128	19	318.8		238.3		80.5		31		32.9	
CK60 x PI229828-F2:3	74	1	51	7	301.9	299.4	235.9	234.11	66	65.6	32.7	31.85	36.6	35.76
		2	27	25	294.8		229.6		65.2		31		34.9	
CK60 x PI229828-F2:3	75	1	113	9	336.2	334.8	266.3	268.06	69.9	67.3	23.4	23.95	19.8	20.07
		2	105	14	337.9		273.3		64.6		24.5		20.2	
CK60 x PI229828-F2:3	76	1	148	3	268.5	278.9	206.6	216.39	61.9	62.3	23.8	23.65	22.2	21.74
		2	40	15	278.1		215.4		62.7		23.5		21.2	
CK60 x PI229828-F2:3	77	1	156	3	263.4	256.3	200.6	192.2	62.8	63.6	26	26.95	22.4	24.46
		2	2	18	226.2		161.8		64.4		27.9		26.6	
CK60 x PI229828-F2:3	78	1	28	12	294.8	295.4	240.9	241.51	53.9	53.2	21.6	22.3	19.8	19.96
		2	145	17	300.6		248.1		52.5		23		20.1	
CK60 x PI229828-F2:3	79	1	103	2	356.1	353.9	289	281.9	67.1	72.2	37.4	38.8	36.1	38.03
		2	64	24	352.7		275.4		77.3		40.2		40	
CK60 x PI229828-F2:3	80	1	26	12	272.7	258.8	183.8	172.75	88.9	85.3	34.8	35.85	32.5	35.13
		2	86	26	242.9		161.3		81.6		36.9		37.7	
CK60 x PI229828-F2:3	81	1	104	2	311.1	279.1	239.9	208.68	71.2	70.4	29.3	31.6	26.2	28.61
		2	32	25	248.1		178.5		69.6		33.9		31	
CK60 x PI229828-F2:3	82	1	98	2	309.3	308.6	235.1	235.66	74.2	72.2	24.8	24.2	23.2	23.08
		2	21	21	303.8		233.7		70.1		23.6		23	
CK60 x PI229828-F2:3	83	1	68	4	296.3	290	228	222.24	68.3	68.9	29.5	30.05	27.1	27.42
		2	83	16	293.5		224.1		69.4		30.6		27.7	
CK60 x PI229828-F2:3	84	1	138	13	251	243.5	184.7	177.37	66.3	66.4	18.5	19.4	17.1	18.02
		2	41	15	233.1		166.7		66.4		20.3		18.9	
CK60 x PI229828-F2:3	85	1	86	11	303.7	312.3	227.1	234.57	76.6	76.4	23.9	24.85	18.6	21.26
		2	138	22	326.5		250.4		76.1		25.8		23.9	
CK60 x PI229828-F2:3	86	1	119	9	287.5	298.3	222.6	231.92	64.9	66.6	24.1	26.95	22.8	26.16
		2	76	16	315.7		247.3		68.3		29.8		29.4	
CK60 x PI229828-F2:3	87	1	29	12	290.8	280.6	228	215.97	62.8	64.1	22.6	21.7	20.3	20.43
		2	6	18	257.8		192.5		65.3		20.8		20.7	
CK60 x PI229828-F2:3	88	1	44	6	326.9	316.6	260.2	249.47	66.7	67.2	32.1	31.15	29.9	29.21
		2	25	25	312.3		244.7		67.6		30.2		28.5	
CK60 x PI229828-F2:3	89	1	122	1	320.5	311.8	250	239.93	70.5	71.3	23.3	23.2	22.9	22.74
		2	152	17	313.6		241.5		72.1		23.1		22.5	
CK60 x PI229828-F2:3	90	1	21	8	338.4	331.1	276.6	267.87	61.8	64.7	29.8	29.4	27.5	28.06
		2	58	23	322.3		254.7		67.6		29		28.7	
CK60 x PI229828-F2:3	91	1	141	13	286.7	291.6	229.2	230.26	57.5	61.2	26.2	26.4	25.7	26.81
		2	144	22	302.2		237.4		64.8		26.6		27.9	
CK60 x PI229828-F2:3	92	1	58	7	285.7	260.9	192	182.65	93.7	78.2	40.1	35.25	37.5	33.97
		2	90	26	233.7		171.1		62.6		30.4		30.4	
CK60 x PI229828-F2:3	93	1	27	12	324.5	305.8	255	236.51	69.5	69.1	28.9	26.75	31.1	27.56
		2	39	15	286.5		217.8		68.7		24.6		24	
CK60 x PI229828-F2:3	94	1	83	5	270.9	271.7	215.1	217.16	55.8	54.3	22.9	24.6	18.9	21.35
		2	49	23	262.1		209.4		52.7		26.3		23.8	
CK60 x PI229828-F2:3	95	1	74	5	297.4	306.2	232.4	240.69	65	63.9	25.5	26.8	19.8	23.16
		2	23	21	302.6		239.8		62.8		28.1		26.5	
CK60 x PI229828-F2:3	96	1	151	3	266.6	273.1	205.9	212.95	59.7	60.3	25.2	25.45	24.4	26.2
		2	53	23	264.2		203.3		60.9		25.7		28	

Table A3. (continued)

Pedigree	Entry #	Rep	Plot	Blk	Number of nodes		Leaf length		Leaf width		Number of tillers		Stalk circumference		GDD to anthesis	
					exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	73	1	94	11	11.4	11.7	78.7	78.1	6.8	6.8	0.6	0.4	4.5	4.99	1162	1168.7
		2	128	19	12.2		77.6		6.8		0.2		5.6		1172	
CK60 x PI229828-F2:3	74	1	51	7	11.8	12	80.5	78.5	6.5	6.7	0.4	1.1	5.4	5.33	1200	1227.9
		2	27	25	11.9		76.4		6.8		1.7		5.1		1257	
CK60 x PI229828-F2:3	75	1	113	9	11.8	11.9	78.1	78.7	6.3	6.6	1.8	1.5	4.2	4.49	1318	1288.9
		2	105	14	11.9		79.2		6.9		1.2		4.8		1269	
CK60 x PI229828-F2:3	76	1	148	3	10.7	11.3	75.4	75.6	6.8	7	1.8	1.4	5	4.8	1217	1213.1
		2	40	15	11.8		75.8		7.2		1.1		4.2		1217	
CK60 x PI229828-F2:3	77	1	156	3	10.3	10.5	76.5	77.5	6.3	6.5	0.7	0.8	5	5.14	1200	1194.6
		2	2	18	10.5		78.3		6.6		1.2		5.2		1200	
CK60 x PI229828-F2:3	78	1	28	12	11.4	11.5	73.7	74.6	6.4	6.5	1.1	1.2	5	4.73	1246	1244.9
		2	145	17	11.8		75.6		6.6		1.2		4.7		1233	
CK60 x PI229828-F2:3	79	1	103	2	13	13	84	83.8	6.6	6.6	2.1	1.9	5.3	4.95	1300	1289.2
		2	64	24	13		83.6		6.5		1.7		4.6		1269	
CK60 x PI229828-F2:3	80	1	26	12	11.3	11.3	73.5	73.9	5.2	5.6	0.5	0.6	6.6	6.14	1172	1186.6
		2	86	26	11.4		74.3		6		0.7		5.9		1200	
CK60 x PI229828-F2:3	81	1	104	2	11.1	11.4	79.4	81.5	6.2	6	2.4	1.9	5.4	5.07	1200	1192.9
		2	32	25	11.6		83.7		5.7		1.4		4.6		1200	
CK60 x PI229828-F2:3	82	1	98	2	11.1	10.8	88.1	86.4	5.9	6.1	3.3	2.8	4.8	4.52	1217	1215.9
		2	21	21	10.6		84.9		6.3		2.1		4.2		1217	
CK60 x PI229828-F2:3	83	1	68	4	10.6	10.2	74.7	75	6	6	1.1	1.2	5.1	5	1200	1183.9
		2	83	16	9.9		76.1		5.9		1.1		5.1		1152	
CK60 x PI229828-F2:3	84	1	138	13	9.3	9.01	74.5	77.4	6.8	6.9	1.3	1.8	4.5	4.26	1112	1073.4
		2	41	15	8.7		80.3		7		2.4		3.8		1057	
CK60 x PI229828-F2:3	85	1	86	11	10.1	9.84	83.2	80.8	6.1	6.1	3	2.5	4.2	4.45	1125	1137.3
		2	138	22	9.8		78.5		6.1		1.9		4.7		1152	
CK60 x PI229828-F2:3	86	1	119	9	12.5	12	81.3	80.8	5.4	5.9	2.3	2.2	5	4.7	1351	1318.1
		2	76	16	11.6		80.2		6.3		2.1		4.5		1284	
CK60 x PI229828-F2:3	87	1	29	12	11.3	10.9	73.4	74.6	5.9	6	1.6	2.2	5.2	4.6	1200	1207.2
		2	6	18	10.5		75.7		6		2.8		4.2		1217	
CK60 x PI229828-F2:3	88	1	44	6	11.7	11.4	80.9	81.8	6.1	6.3	2	1.8	5.5	4.97	1217	1234.7
		2	25	25	11		82.4		6.4		1.6		4.4		1257	
CK60 x PI229828-F2:3	89	1	122	1	10.6	10.6	78.5	77.5	7	7.1	1.4	1.8	4.7	4.92	1152	1131.4
		2	152	17	10.7		76.7		7.2		2.1		5		1112	
CK60 x PI229828-F2:3	90	1	21	8	10.9	10.9	74.4	76.7	6.2	6.4	0.2	0.6	4.9	4.76	1217	1214.1
		2	58	23	10.8		79		6.6		1.2		4.3		1200	
CK60 x PI229828-F2:3	91	1	141	13	10.4	9.93	72.9	75.7	6	6	2.5	2.1	5	4.75	1200	1169.2
		2	144	22	9.6		78.5		6		1.6		4.7		1152	
CK60 x PI229828-F2:3	92	1	58	7	10.8	10.9	85.2	84	7.3	7.2	1.1	1.1	6.8	6.48	1217	1278
		2	90	26	10.9		82.8		7.1		1.2		6.3		1334	
CK60 x PI229828-F2:3	93	1	27	12	13.5	12.9	79.2	80	5.9	5.9	1.1	1.6	5.3	4.91	1233	1225.2
		2	39	15	12.3		81		5.9		2.1		4.4		1217	
CK60 x PI229828-F2:3	94	1	83	5	12.3	11.5	74	71.1	6.8	6.9	0.5	0.7	4.7	4.65	1300	1284.6
		2	49	23	10.6		68.2		7		0.8		4.1		1284	
CK60 x PI229828-F2:3	95	1	74	5	11.1	10.9	82.1	83.6	6.2	6.5	1	1.3	5	5.17	1246	1225.2
		2	23	21	10.8		85.2		6.7		1.3		5.1		1217	
CK60 x PI229828-F2:3	96	1	151	3	10.2	10.1	74.2	75.1	6.5	6.4	1.5	1.6	4.9	4.71	1200	1166.8
		2	53	23	9.7		75.9		6.3		1.8		4		1137	

Table A3. (continued)

Pedigree	Entry #	Rep	Plot	Blk	Plant height		Plant height to the uppermost node		Peduncle length		Panicle length		Panicle width	
					exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	97	1	22	8	292.4	268.3	224.6	203.36	67.8	65.5	33.2	30.75	33	31.3
		2	151	17	252		188.8		63.2		28.3		29.6	
CK60 x PI229828-F2:3	98	1	105	2	332.3	321.5	241.8	237.12	90.5	84	31.9	29.25	30.1	26.97
		2	146	17	317.9		240.4		77.5		26.6		23.8	
CK60 x PI229828-F2:3	99	1	136	13	255.1	266.7	184	197.67	71.1	69.7	30.1	30.15	30	29.99
		2	59	23	271.3		203.1		68.2		30.2		30	
CK60 x PI229828-F2:3	100	1	96	11	293.8	279.5	237.1	223.63	56.7	55.3	24.5	24.9	24.4	25.84
		2	54	23	258		204.2		53.8		25.3		27.3	
CK60 x PI229828-F2:3	101	1	20	8	340.6	327.9	272.8	261.66	67.8	67.4	31.6	29	34	31.64
		2	38	15	317.9		251		66.9		26.4		29.3	
CK60 x PI229828-F2:3	102	1	62	4	329.3	324.5	265.3	263.19	64	61.9	28.5	27.5	27.5	26.47
		2	33	25	320.6		260.8		59.8		26.5		25.4	
CK60 x PI229828-F2:3	103	1	65	4	279.2	277.4	207.3	208.47	71.9	70.2	24.3	24.8	27.9	27.7
		2	126	19	275.2		206.7		68.5		26.7		27.6	
CK60 x PI229828-F2:3	104	1	8	10	309.4	297.3	236.3	223.86	73.1	74.2	27.6	28.55	27.6	27.62
		2	47	15	294.2		218.9		75.3		29.5		27.6	
CK60 x PI229828-F2:3	105	1	128	1	276.1	273.1	194.6	190.68	81.5	82.5	32.5	33.95	29.7	33.04
		2	44	15	275.6		192.2		83.4		35.4		36.3	
CK60 x PI229828-F2:3	106	1	124	1	292.6	288.4	231.5	224.38	61.1	63.6	22.9	23.15	19.3	19.82
		2	142	22	298.2		232.1		66.1		23.4		20.3	
CK60 x PI229828-F2:3	107	1	155	3	276.9	292.3	213.6	227.59	63.3	64.4	27.7	28.2	26.8	27.04
		2	31	25	295.5		230.1		65.4		28.7		27.2	
CK60 x PI229828-F2:3	108	1	55	7	311.1	314.2	245.9	249.17	65.2	65.9	26.2	26	25.1	25.97
		2	51	23	312.1		245.5		66.6		25.8		26.9	
CK60 x PI229828-F2:3	109	1	49	7	280.3	275.7	219.1	214.66	61.2	61.8	27.9	29.15	29	29.61
		2	84	16	277.8		215.4		62.4		30.4		30.2	
CK60 x PI229828-F2:3	110	1	10	10	309.9	287.1	237.7	218.09	72.2	69.9	21	21.5	19.2	19.94
		2	71	24	272.4		204.8		67.6		22		20.7	
CK60 x PI229828-F2:3	111	1	120	9	294.1	289.1	217	214.06	77.1	74.5	26.4	27.5	20.7	23.42
		2	155	17	288.1		216.2		71.9		28.6		26	
CK60 x PI229828-F2:3	112	1	16	8	316	312.4	242.5	242.09	73.5	71.9	28.5	27.55	29.8	29.03
		2	131	19	309		238.8		70.2		26.6		28.4	
CK60 x PI229828-F2:3	113	1	80	5	308.6	304	248.6	237.66	60	66	23.5	23.05	21.1	21.29
		2	81	16	300.9		229		71.9		22.6		21.4	
CK60 x PI229828-F2:3	114	1	50	7	269.4	278.6	203.6	209.96	65.8	69.7	33	33.8	40.7	39.76
		2	107	14	292.4		218.9		73.5		34.6		38.8	
CK60 x PI229828-F2:3	115	1	9	10	318	296.8	241.4	223.36	76.6	74.8	24.5	23.65	23.3	23.58
		2	102	14	290.3		217.4		72.9		22.8		23.8	
CK60 x PI229828-F2:3	116	1	126	1	187.4	174.2	110.7	99.08	76.7	74.7	28	28.5	30.3	30.57
		2	118	20	165.2		92.5		72.7		29		30.9	
CK60 x PI229828-F2:3	117	1	105	2	260.7	245.4	176.6	167.27	84.1	77.8	34.1	32.35	31.7	30.94
		2	140	22	240.6		169.1		71.5		30.6		30.2	
CK60 x PI229828-F2:3	118	1	66	4	320.4	314.9	262.6	255.68	57.8	59.6	30	29.75	29	28.85
		2	143	22	320		258.6		61.4		29.5		28.7	
CK60 x PI229828-F2:3	119	1	63	4	258.8	252	210	203.17	48.8	49.7	20.6	22.4	18.8	20.68
		2	67	24	246.2		195.6		50.6		24.2		22.6	
CK60 x PI229828-F2:3	120	1	70	4	297.8	287.7	212.9	209.24	84.9	79.2	26.2	28.05	25.3	27.62
		2	46	15	279.5		206		73.5		29.9		29.9	

Table A3. (continued)

Pedigree	Entry			Number of nodes		Leaf length		Leaf width		Number of tillers		Stalk circumference		GDD to anthesis		
	#	Rep	Plot	Blk	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.		
CK60 x PI229828-F2:3	97	1	22	8	11.5	11	78.6	79.2	6.9	6.7	1.1	0.9	6.2	5.87	1162	1165.5
		2	151	17	10.6		80		6.5	0.8			5.7		1152	
CK60 x PI229828-F2:3	98	1	105	2	10.9	11.1	80.8	77.2	6.8	6.8	1.2	1.1	5.2	5.11	1112	1137.8
		2	146	17	11.5		73.8		6.7	1			5.2		1162	
CK60 x PI229828-F2:3	99	1	136	13	10.4	10.5	77.1	76.9	6.5	6.8	1.4	1.1	5.9	5.37	1152	1143
		2	59	23	10.4		76.8		7.1	1			4.5		1152	
CK60 x PI229828-F2:3	100	1	96	11	11.3	10.7	76	77.1	6.6	6.8	0.1	0.2	4.8	4.96	1200	1177.6
		2	54	23	10		78.2		7	0.3			4.6		1162	
CK60 x PI229828-F2:3	101	1	20	8	11.6	11.2	79.3	80.8	7.2	7.6	1.1	1.1	5.3	4.85	1217	1234.9
		2	38	15	10.7		82.4		7.9	1.2			4.2		1246	
CK60 x PI229828-F2:3	102	1	62	4	12.4	12.1	80.4	79	6.6	6.5	0.7	1.1	5.6	5.29	1334	1331
		2	33	25	11.7		77.5		6.4	1.4			4.8		1334	
CK60 x PI229828-F2:3	103	1	65	4	10.5	10.3	72.1	71.6	7.2	7.3	0.3	0.5	5.3	5.2	1152	1183
		2	126	19	10.2		71.1		7.4	0.5			5.4		1200	
CK60 x PI229828-F2:3	104	1	8	10	10	10.3	79.4	81.8	5.3	5.6	1.6	1.7	4.3	4.17	1152	1130.7
		2	47	15	10.6		84.2		5.9	1.8			3.8		1112	
CK60 x PI229828-F2:3	105	1	128	1	10.7	10.6	78.2	79.1	6.3	6.6	1.6	1.4	5.2	5.45	1200	1180.2
		2	44	15	10.4		80.2		6.9	1.3			5.2		1172	
CK60 x PI229828-F2:3	106	1	124	1	10.3	10.7	76.6	75.9	5.1	5.6	1.9	1.6	4.3	4.59	1200	1198.6
		2	142	22	11.1		75.3		6.1	1.2			4.8		1200	
CK60 x PI229828-F2:3	107	1	155	3	10.9	11.1	70	70.2	6.6	7	0.8	0.7	5.1	5.27	1162	1174.4
		2	31	25	11.2		70.3		7.3	0.6			5.1		1200	
CK60 x PI229828-F2:3	108	1	55	7	11	11.1	79.2	79.8	7	7	2.5	2.2	5.6	5.36	1200	1204.3
		2	51	23	10.9		80.4		7	1.8			4.8		1200	
CK60 x PI229828-F2:3	109	1	49	7	10.2	10.6	74.4	74.3	6.1	6.3	1.1	1.1	4.8	4.73	1152	1186.2
		2	84	16	11		74.2		6.4	1.1			4.9		1160	
CK60 x PI229828-F2:3	110	1	10	10	10.8	10.3	71.9	74.5	6.4	6.7	1.3	1.4	5	4.47	1162	1151.3
		2	71	24	9.9		76.9		7	1.5			3.9		1125	
CK60 x PI229828-F2:3	111	1	120	9	10.9	11.4	70.5	72.5	6.2	6.7	1.3	0.8	5.2	5.34	1217	1192.1
		2	155	17	12		74.7		7.1	0.4			5.5		1172	
CK60 x PI229828-F2:3	112	1	16	8	11.1	11	74.6	75.3	6.1	6.6	1.4	1.1	4.8	4.58	1152	1162.7
		2	131	19	10.9		76.1		7	0.7			4.7		1152	
CK60 x PI229828-F2:3	113	1	80	5	11.8	11.4	73.2	75.3	6.7	6.9	0.7	0.8	5.3	5.52	1217	1183.1
		2	81	16	11.2		77.4		7.1	0.8			5.8		1152	
CK60 x PI229828-F2:3	114	1	50	7	11	11	83.8	82.1	6.2	6.3	1.3	1.7	5.6	5.47	1257	1250
		2	107	14	10.8		80.4		6.3	1.9			5.5		1233	
CK60 x PI229828-F2:3	115	1	9	10	11.1	11	75.8	75.3	5.4	5.7	1	0.9	5.4	5.09	1162	1168.7
		2	102	14	10.9		74.7		6	0.6			4.9		1172	
CK60 x PI229828-F2:3	116	1	126	1	10.6	10	71.7	75.4	6.3	6.9	0.4	0.4	6	6.07	1184	1172
		2	118	20	9.2		79.2		7.4	0.4			6.1		1162	
CK60 x PI229828-F2:3	117	1	106	2	8.7	8.86	84.5	82.8	6.6	6.8	1	1.3	5.1	4.94	1057	1057
		2	140	22	9.1		81.2		6.9	1.4			5		1057	
CK60 x PI229828-F2:3	118	1	65	4	10.9	11	81.2	79.4	5.9	5.8	1.3	1.6	5.1	4.81	1200	1204.1
		2	143	22	11.2		77.5		5.6	1.6			4.7		1200	
CK60 x PI229828-F2:3	119	1	63	4	10.4	10.4	73.1	75.4	6.4	6.5	1.6	1.7	5.2	5.08	1217	1203.3
		2	67	24	10.5		77.7		6.6	1.8			4.9		1172	
CK60 x PI229828-F2:3	120	1	70	4	8.6	8.56	80	79.9	6.5	7	1.6	1.7	5.2	5.02	1057	1056.7
		2	46	15	8.5		79.9		7.4	1.7			4.6		1057	

Table A3. (continued)

Pedigree	Entry #	Rep	Plot	Blk	Plant height		Plant height to the uppermost node		Peduncle length		Panicle length		Panicle width	
					exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	121	1	97	2	235.5	221	159.8	146.37	75.7	74.4	30.6	31.3	26.4	30.35
		2	117	20	207.4		134.3		73.1		31.8		34.4	
CK60 x PI229828-F2:3	122	1	14	8	154.5	162	87.8	99.64	66.7	63.5	28	29.5	32.1	34.26
		2	70	24	171.1		110.8		60.3		31		36.5	
CK60 x PI229828-F2:3	123	1	32	12	294.7	286.5	240	230.47	54.7	56.3	23.5	22.2	23.7	22.07
		2	56	23	273.6		215.8		57.8		20.9		20.5	
CK60 x PI229828-F2:3	124	1	112	9	286.4	254.9	220.2	192.49	66.2	62.6	26.6	25.65	28.1	27.12
		2	72	24	221.4		162.5		58.9		24.7		26.1	
CK60 x PI229828-F2:3	125	1	123	1	277.7	269.3	212.1	207.56	65.6	61.8	24	24.1	25.4	26.1
		2	66	24	265.3		207.3		58		24.2		26.8	
CK60 x PI229828-F2:3	126	1	146	3	220.1	214.4	130.6	130.88	89.5	83	30.5	31.8	33.7	34.37
		2	115	20	196.4		120		76.4		33.1		35.1	
CK60 x PI229828-F2:3	127	1	135	13	302.7	283.6	227.9	212.39	74.8	71.5	26.5	25.65	25.3	25.14
		2	65	24	260.5		192.4		68.1		24.8		25	
CK60 x PI229828-F2:3	128	1	142	13	316.9	321.9	246.9	255.27	70	66.7	31.5	32.15	31.5	32.33
		2	29	25	323		259.6		63.4		32.8		33.1	
CK60 x PI229828-F2:3	129	1	15	8	302.1	295.1	229.7	229.46	72.4	66.3	25.6	25.6	31	29.83
		2	139	22	299.2		239.1		60.1		25.6		28.7	
CK60 x PI229828-F2:3	130	1	60	7	241.5	226.4	160.4	148.94	81.1	78.5	29.6	28.2	30.2	30.59
		2	122	19	207.9		132.1		75.8		26.8		31.1	
CK60 x PI229828-F2:3	131	1	67	4	318.2	294	239	214.93	79.2	79.3	28.9	28.35	26.1	25.94
		2	95	26	270.4		191.1		79.3		27.8		25.7	
CK60 x PI229828-F2:3	132	1	127	1	261.5	245.8	185.8	173.19	75.7	72.5	25.8	25.3	24.7	24.39
		2	36	25	234.5		165.3		69.2		24.8		24	
CK60 x PI229828-F2:3	133	1	52	7	295.2	290.8	207.1	203.92	88.1	87.1	31.9	34	30.7	33.83
		2	1	18	273.5		187.5		86		36.1		37.1	
CK60 x PI229828-F2:3	134	1	33	12	312.6	297.2	231.7	220.54	80.9	76.9	27.8	28.1	30.8	32.94
		2	132	19	278.7		205.8		72.9		28.4		35.2	
CK60 x PI229828-F2:3	135	1	19	8	308.2	304.5	239.7	236.37	68.5	69	30.8	31	35.3	35.25
		2	26	25	302.3		232.8		69.5		31.2		35.2	
CK60 x PI229828-F2:3	136	1	147	3	323.3	339.3	261.7	276.22	61.6	63.4	28.7	30.5	23.3	24.72
		2	121	19	341.7		276.6		65.1		32.3		26.2	
CK60 x PI229828-F2:3	137	1	72	4	301.7	289.4	225.1	214.25	76.6	76.3	26.3	26.8	23.1	24.18
		2	52	23	274.9		198.9		76		27.3		25.3	
CK60 x PI229828-F2:3	138	1	149	3	280.3	294.1	214	228.17	66.3	65.8	25.9	26.2	23.2	22.15
		2	63	24	295.8		230.4		65.3		26.5		21.1	
CK60 x PI229828-F2:3	139	1	17	8	285.6	270.3	202.4	192.41	83.2	78.6	25.4	30.05	24.6	28.83
		2	112	20	266.5		182.5		74		34.7		33.2	
CK60 x PI229828-F2:3	140	1	137	13	317.5	280.7	245.1	209.76	72.4	70.3	25.6	26.75	29.2	30.8
		2	18	21	235		166.8		68.2		27.9		32.4	
CK60 x PI229828-F2:3	141	1	145	3	286	285.7	230.3	228.98	55.7	55.9	25.7	25.6	25.4	25.46
		2	96	26	272.9		216.7		58.1		25.5		25.4	
CK60 x PI229828-F2:3	142	1	140	13	268.3	254.6	205.2	197.44	62.1	67.9	23	23.65	21.3	22.51
		2	127	19	255.6		182		73.6		24.3		23.8	
CK60 x PI229828-F2:3	143	1	48	6	277.8	269	219.6	210.66	58.2	58.1	26	25.4	24.8	26.14
		2	114	20	266		208		58		24.8		27.6	
CK60 x PI229828-F2:3	144	1	131	1	281.5	267.5	211.1	196.99	70.4	70.2	26.5	26.05	23.2	24.26
		2	5	18	247.1		177.1		70		25.6		25.4	

Table A3. (continued)

Pedigree	Entry			Number of nodes		Leaf length		Leaf width		Number of tillers		Stalk circumference		GDD to anthesis		
	#	Rep	Plot	Blk	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	121	1	97	2	11.2	10.8	80	79.5	7.4	7.8	0.7	0.5	5.9	5.86	1217	1184.9
		2	117	20	10.2		79.1		8.2		0.3		6.1		1152	
CK60 x PI229828-F2:3	122	1	14	8	10.1	10.8	75.1	77.9	5.4	6.2	0.1	0	5.9	5.9	1172	1174.4
		2	70	24	11.5		80.8		7		0.1		5.9		1152	
CK60 x PI229828-F2:3	123	1	32	12	10.5	10.3	75.4	77.4	6.4	6.7	1.2	1.5	5.1	4.87	1172	1188.4
		2	56	23	9.9		79.4		7		1.7		4.4		1200	
CK60 x PI229828-F2:3	124	1	112	9	10.1	9.87	75.8	75	7.1	7.2	2.1	1.8	4.7	4.72	1152	1106
		2	72	24	9.7		74.2		7.2		1.7		4.6		1057	
CK60 x PI229828-F2:3	125	1	123	1	11.7	12.1	80	79.7	6	6.3	0.9	1.3	4.9	5.11	1284	1279.8
		2	66	24	12.5		79.5		6.6		1.7		5		1269	
CK60 x PI229828-F2:3	126	1	146	3	9.8	9.29	90	88.5	5.7	5.9	0.9	0.9	5.2	5.21	1172	1162.8
		2	115	20	8.5		86.9		6.1		1		5.3		1152	
CK60 x PI229828-F2:3	127	1	135	13	10.4	10.2	81	76.7	6.1	6.3	0.7	0.9	4.6	4.61	1152	1116.4
		2	65	24	10		72.4		6.4		1.2		4.6		1085	
CK60 x PI229828-F2:3	128	1	142	13	11.6	11.6	75.7	75.4	5.9	6.2	1.5	1.2	4.9	4.63	1200	1186.2
		2	29	25	11.5		75		6.5		0.9		4.2		1200	
CK60 x PI229828-F2:3	129	1	15	8	11.7	12	81.2	78.6	6.8	6.6	1.8	1.5	5.7	5.39	1217	1239.2
		2	139	22	12.5		76.1		6.3		1.2		5.3		1246	
CK60 x PI229828-F2:3	130	1	60	7	8.3	8.9	81.2	81.8	6.6	6.9	1	0.9	5.5	5.34	1112	1100.4
		2	122	19	9.4		82.4		7.2		0.5		5.5		1070	
CK60 x PI229828-F2:3	131	1	67	4	10.3	10	77.9	78.3	6.7	7	0.2	1	5.8	5.69	1152	1152.1
		2	95	26	9.8		78.6		7.2		1.8		5.7		1152	
CK60 x PI229828-F2:3	132	1	127	1	10.8	10.2	83.7	83.3	7	6.8	2.1	1.7	5.5	5.38	1246	1223
		2	36	25	9.5		82.9		6.6		1.2		4.8		1217	
CK60 x PI229828-F2:3	133	1	52	7	10.2	10.5	79.1	79.3	6.9	7	1.1	1.8	5.4	5.19	1112	1125.1
		2	1	18	10.5		79.5		7.1		2.5		5.1		1137	
CK60 x PI229828-F2:3	134	1	33	12	10.4	10.3	74.4	74.6	7.3	7.5	1.2	1	5.1	4.9	1112	1092.1
		2	132	19	10.3		74.9		7.6		0.7		5.1		1057	
CK60 x PI229828-F2:3	135	1	19	8	11.8	11.8	72.1	73.2	7	7.2	0.6	0.5	5.9	5.77	1162	1190.2
		2	26	25	11.7		74.3		7.3		0.5		5.5		1217	
CK60 x PI229828-F2:3	136	1	147	3	12.4	12.4	71.1	72.5	6.8	6.6	1.2	1	4.9	5.08	1246	1226.4
		2	121	19	12.3		73.7		6.3		0.8		5.4		1200	
CK60 x PI229828-F2:3	137	1	72	4	10	10.1	78.6	80.5	5.9	6.4	0.5	1.1	4.7	4.68	1152	1153.9
		2	52	23	10.1		82.3		6.8		1.7		4.3		1152	
CK60 x PI229828-F2:3	138	1	149	3	10.9	11.3	85.6	85.7	6.6	6.6	1.8	1.6	5.1	4.85	1269	1274.1
		2	63	24	11.7		85.6		6.6		1.6		4.4		1269	
CK60 x PI229828-F2:3	139	1	17	8	11.3	10.9	74.2	73.7	6.8	7.1	0.1	0.3	5.9	5.96	1172	1210.6
		2	112	20	10.3		73.1		7.4		0.7		6.3		1233	
CK60 x PI229828-F2:3	140	1	137	13	11.3	10.6	73.2	74.8	5.9	6.6	1.9	1.6	5.1	5.08	1200	1148.1
		2	18	21	10		76.4		7.3		1.2		5		1112	
CK60 x PI229828-F2:3	141	1	145	3	11.8	11.6	80.9	83.4	6.2	6.1	1.6	2.1	4.9	5.07	1351	1339
		2	96	26	11.4		85.8		6		2.8		5.2		1334	
CK60 x PI229828-F2:3	142	1	140	13	9.7	9.47	73.6	74.5	6.4	7	2.3	1.7	4.8	4.49	1152	1100.7
		2	127	19	9.3		75.4		7.5		1		4.5		1057	
CK60 x PI229828-F2:3	143	1	48	6	10.3	10.6	78	78.8	6.5	6.8	0.8	1.2	4.4	4.36	1137	1154.7
		2	114	20	10.7		79.3		7		1.7		4.7		1162	
CK60 x PI229828-F2:3	144	1	131	1	10	9.99	72.4	72.7	5.8	6.3	2	2.4	4.3	4.59	1172	1147.2
		2	5	18	9.9		72.9		6.8		2.9		4.7		1137	

Table A3. (continued)

Pedigree	Entry #	Rep	Plot	Blk	Plant height		Plant height to the uppermost node		Peduncle length		Panicle length		Panicle width	
					exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	145	1	30	12	309	312.3	238.4	238.6	70.6	73.1	31.9	32.5	34.6	34.44
		2	119	20	313.8		238.2		75.6		33.1		34.4	
CK60 x PI229828-F2:3	146	1	4	10	330.6	317.1	255.8	244.56	74.8	73.2	31.1	31	31.1	31.58
		2	28	25	311.6		240.1		71.5		30.9		32	
CK60 x PI229828-F2:3	147	1	24	8	305.7	285.8	233.4	219.26	72.3	67.1	25.2	23.05	22.4	21.06
		2	91	26	267.2		205.4		61.8		20.9		19.7	
CK60 x PI229828-F2:3	148	1	110	9	336.2	331.8	252.2	247.84	84	84.5	40.7	41.2	40	43.59
		2	124	19	323.9		239		84.9		41.7		47.2	
CK60 x PI229828-F2:3	149	1	87	11	313.8	304.3	234.7	222.82	79.1	80.1	32	31.15	30.6	30.57
		2	113	20	290.6		209.5		81.1		30.3		30.6	
CK60 x PI229828-F2:3	150	1	34	12	326.1	312.5	243.8	232.54	82.3	79.9	31.1	31.65	31.7	34.92
		2	62	24	297.3		219.9		77.4		32.2		38.2	
CK60 x PI229828-F2:3	151	1	3	10	262	237.1	186	157.05	76	79.9	31.9	32	33.2	34.14
		2	17	21	215.1		131.3		83.8		32.1		35.1	
CK60 x PI229828-F2:3	152	1	144	13	318	319.5	257	257.57	61	61.8	35	34.75	37.4	37.8
		2	11	18	306.1		243.6		62.5		34.5		38.3	
CK60	153	1	45	6	89.8	91.13	40.7	39.41	49.1	51.3	21.8	22.4	19.8	19.42
		2	94	26	98.1		44.6		53.5		23		19	
PI229828	154	1	56	7	314	313.5	246.9	244.74	67.1	69.3	34.8	34.75	32	33.12
		2	69	24	310.9		239.4		71.5		34.7		34.3	
CK60 x PI229828-F1	155	1	125	1	328.5	298.4	247.9	220.99	80.6	77.7	32.6	30.7	32.8	31.49
		2	79	16	281.5		206.7		74.8		28.8		30.1	
CK60 x PI229828-F1	156	1	82	5	331	312.8	259.4	241.65	71.6	70.3	32.8	29.7	31.1	28.7
		2	30	25	287.3		218.3		69		26.6		26.2	

Blk = block in lattice design; exp. = experiment plot value; adj. = adjusted entry mean value.



Table A3. (continued)

Pedigree	Entry #	Rep	Plot	Blk	Number of nodes		Leaf length		Leaf width		Number of tillers		Stalk circumference		GDD to anthesis	
					exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.	exp.	adj.
CK60 x PI229828-F2:3	145	1	30	12	10.8	11.2	82.3	81.6	6	6.1	1.2	0.9	5	4.78	1152	1162
		2	119	20	11.4		80.9		6.1		0.6		4.9		1162	
CK60 x PI229828-F2:3	146	1	4	10	12.2	11.6	76.5	79.3	7.1	7.2	1	0.9	5.6	5.54	1200	1177
		2	28	25	11		82		7.2		0.7		5.3		1162	
CK60 x PI229828-F2:3	147	1	24	8	10.9	10.8	75.2	73.4	6.7	6.9	0.8	0.7	5.4	5.17	1112	1135.8
		2	91	26	10.7		71.6		7.1		0.7		5.1		1152	
CK60 x PI229828-F2:3	148	1	110	9	11.3	11.4	84.1	84.3	7.3	7.6	1.4	1.5	6.2	6.1	1246	1239.3
		2	124	19	11.5		84.6		7.8		1.5		6.2		1233	
CK60 x PI229828-F2:3	149	1	87	11	10.2	9.74	82.1	81	6.2	6.4	1.1	1.5	4.5	4.47	1112	1103.1
		2	113	20	9.2		80		6.6		2		4.5		1096	
CK60 x PI229828-F2:3	150	1	34	12	9.9	10.2	74	72.6	6.8	7.5	1.6	1.5	6	5.57	1057	1066.3
		2	62	24	10.5		71.3		8.2		1.3		5.2		1057	
CK60 x PI229828-F2:3	151	1	3	10	11	10.8	73.5	74.9	6.8	7.3	0.4	0.9	5.8	5.54	1152	1154
		2	17	21	10.8		76.2		7.8		1.1		5.2		1152	
CK60 x PI229828-F2:3	152	1	144	13	11.2	11	79.7	80.4	7.2	7.6	2	1.6	5.5	5.55	1246	1218.9
		2	11	18	10.8		81		7.9		1.5		5.7		1217	
CK60	153	1	45	6	9.9	10.6	65.2	67.4	6.3	6.8	0.1	0.1	7.1	7.07	1246	1223.8
		2	94	26	11.3		69.4		7.3		0.3		7.3		1200	
PI229828	154	1	56	7	11.1	10.7	80.9	83.4	5.5	5.7	1.7	2.4	4.7	4.41	1217	1219.6
		2	69	24	10.2		86		5.8		3		4.1		1200	
CK60 x PI229828-F1	155	1	125	1	11.7	11.3	83.9	81.3	6.8	6.6	0.9	1.1	4.6	5.13	1172	1164.4
		2	79	16	11		78.6		6.4		1.3		5.6		1152	
CK60 x PI229828-F1	156	1	82	5	11.1	10.7	79.8	77.8	6.8	6.9	1.5	1.7	5.1	4.92	1172	1149.7
		2	30	25	10.3		75.8		6.9		1.8		4.4		1152	

Blk = block in lattice design; exp. = experiment plot value; adj. = adjusted entry mean value.

Table A4. Analysis of variance for morphological traits.

Source	df	Plant height	Height to the uppermost node	Peduncle length	Number of nodes	Panicle length	Panicle width
Replications	1	18688.4**	13917.4**	351.6**	0.73	2.8	55.4**
Treatments (unadjusted)	155	2942.5**	2872.8**	127.4**	1.52**	33.9**	53.0**
Treatments(adjusted)	155	2932.2**	2876.9**	127.4**	1.49**	33.9**	52.9**
Lines	151	2483.8**	2513.2**	125.8**	1.52**	33.7**	52.8**
Checks	3	23660.8**	19505.5**	251.3**	0.22	52.9**	74.9**
Parents	1	49430.6**	42160.4**	324.0**	0.00	152.5**	187.7**
F1	1	207.9	426.8	54.8	0.39	1	7.7
Parents vs. F1	1	2134.8**	15929.3**	375.4**	0.25	5.2	29.2**
Lines vs. Checks	1	8443.4**	7914.3**	10.3	0.33	4.6	0.7
Blocks Adjusted	24	558.6**	510.1**	18.5	0.32*	1.8	4.9
Intrablock Error	131	233.0	217.3	19.8	0.21	3.7	4.7
RCB Error	155	283.5	262.7	19.6	0.21	3.4	4.7
Effective Error	131	254.2	236.7	19.6	0.21	3.4	4.7
Total	311						
Experimental Mean		286.0	217.7	68.03	11.0	28.6	27.9
LSD (0.05)		31.5	30.4	8.8	0.91	3.6	4.3
CV (%)		5.5	7.0	6.4	4.1	6.4	7.8
Efficiency (%)		111.5	111.0	100.0	103.3	100.0	100.1

Significance levels of probability for mean squares = \*0.05, \*\*0.01.

Table A4. (continued)

Source	df	Leaf length	Leaf width	Stalk circumference	Number of tillers	GDD to anthesis
Replications	1	45.3**	8.9**	4.5**	1.1*	7473.5**
Treatments (unadjusted)	155	37.7**	0.49**	0.54**	0.60**	7409.4**
Treatments(adjusted)	155	37.6**	0.49**	0.48**	0.58**	7353.1**
Lines	151	36.6**	0.49**	0.44**	0.56**	7486.8**
Checks	3	101.1**	0.01	2.7**	1.8**	2865.9**
Parents	1	257.6**	1.32**	7.0**	5.0**	17.8
F1	1	12.1	0.06	0.04	0.37	216.9
Parents vs. F1	1	33.7**	0.50*	1.0**	0.04	8363.1**
Lines vs. Checks	1	1.4	0.09	0.68**	0.00	18.5
Blocks Adjusted	24	5.8	0.09	0.29**	0.33*	1263.3**
Intrablock Error	131	5.4	0.09	0.07	0.21	546.8
RCB Error	155	5.5	0.09	0.07	0.23	657.8
Effective Error	131	5.5	0.09	0.08	0.22	595.1
Total	311					
Experimental Mean		77.0	6.60	5.1	1.33	1187.8
LSD (0.05)		4.6	0.62	0.57	0.94	48.3
CV (%)		3.0	4.74	5.6	35.7	2.0
Efficiency (%)		100.1	100.1	130.8	103.1	110.5

Significance levels of probability for mean squares = \*0.05, \*\*0.01.

Table A5. Precipitation amounts and GDD accumulations site averages and deviations from the site average per month in the growing season of 1992 and 1994.

	Precipitation (mm)			GDD Accumulation (°C)		
	Average†	Deviations in year		Average†	Deviations In year	
		1992	1994		1992	1994
May	111	-85	-67	225	21	8
June	130	-115	12	330	-2	24
July	88	172	-30	400	-71	-43
Aug.	99	-42	14	363	-82	-30
Sept.	82	22	32	247	2	25
May-Sept.	509	-48	-39	1565	-133	-16

†Site average based on data from 1951-1980

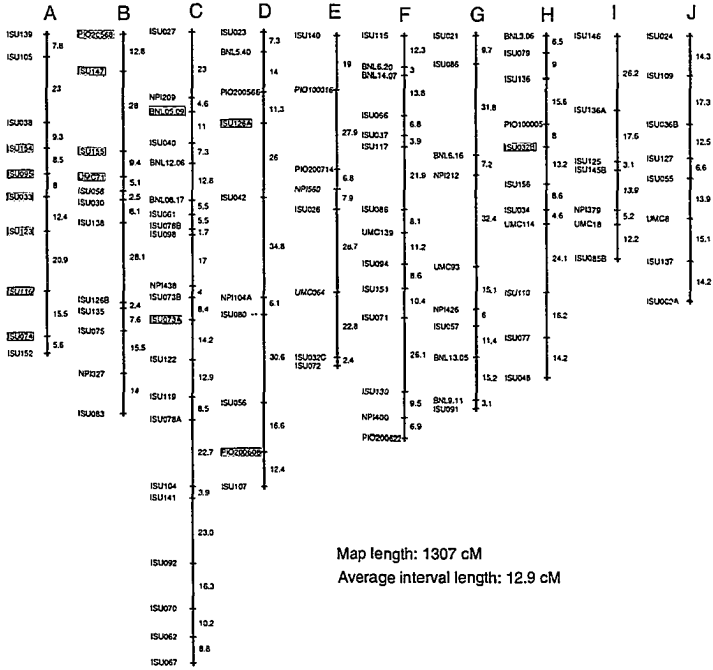


Figure A1. RFLP linkage map of sorghum  $F_2$  population CK60 versus PI229828. Loci with distorted Mendelian segregation ratio are marked with open boxes. The numbers to the right of each linkage group represent the map distance in cM.